Research Article

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Nutrient-efficient catfish-based aquaponics for producing lamb's lettuce at two light intensities

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Abstract

BACKGROUND: Aquaponic systems are sustainable processes of managing water and nutrients for food production. An innovate nutrient-efficient catfish-based (*Clarias gariepinus*) aquaponics system was implemented for producing two cultivars of two leafy vegetables largely consumed worldwide: lamb's lettuce (*Valerianella locusta* var. Favor and *Valerianella locusta* var. de Hollande) and arugula (*Eruca vesicaria* var. sativa and *Eruca sativa*). Different growing treatments (4 × 2 factorial design) were applied to plants of each cultivar, grown at two light intensities (120 and 400 μmol m^{-2} s^{-1}). During growth, several morphological characteristics (root length, plant height, leaf number, foliage diameter and biggest leaf length) were measured. At harvest, plants were weighed and examined qualitatively in terms of greenness and health status. Additionally, leaf extracts were obtained and used to determine total phenolic contents, antioxidant capacities, and levels of cytotoxicity to Caco-2 intestinal model cells.

RESULTS: After a 5-week growth period, both lamb's lettuce cultivars presented high levels of greenness and health status, at both light intensities, particularly the var. de Hollande that also showed higher average performance in terms of plant morphology. In turn, arugula cultivars showed lower levels of greenness and health status, especially the cultivar *E. vesicaria* var. sativa submitted to direct sunlight during growth. In addition, plant specimens submitted to higher levels of light intensity showed higher contents in antioxidants/polyphenols. Cultivars with a higher content in antioxidants/polyphenols led to higher Caco-2 cell viability.

CONCLUSION: For successful industrial implementation of the aquaponics technology, different and optimized acclimatizing conditions must be applied to different plant species and cultivars.

Supporting information may be found in the online version of this article.

Keywords: aquaponics; *Clarias gariepinus*; *Eruca sativa*; nutrient management; plant growth and development; *Valerianella locusta*

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INTRODUCTION

Global water resources are under increasing pressure. Population growth, economic development, urbanization and intensified agricultural production have generated a substantial rise in water demand, with consumption and degradation levels constantly exceeding regeneration rates.[1] Besides other natural resources (arable lands, fossil energy and nutrients), increasing food demands deeply rely on freshwater availability and renewability. Agriculture is one of the most water consuming activities, accounting for 70% of the world's needs for freshwater, invested in the production of around 40% of food worldwide.[2] Expansion and intensification of crop production on irrigated soil is the most significant driver of water demand, along with agro-forestry, livestock water storage and aquaculture. Therefore, it is paramount to implement and develop safe reuse practices. Cleaner practices, such as sustainable aquaponics, can be used simultaneously for food production and wastewater recovery.[3]

Aquaponics is a type of integrated multitrophic system that combines two food production systems, namely, aquaculture and hydroponics, in a more sustainable and symbiotic environment, where nutrients and water are kept in recirculation between subsystems.[4] Various commercial and specialty crops can be grown using aquaponics, including leafy vegetables, tomatoes, cucumbers, peppers and strawberries.[5-8] Aquaponic systems are a promising solution to feed the growing population and provide significant opportunities, at the same time as reducing freshwater usage and improving waste management and nutrient recycling.[5] Fish waste, in the form of nitrogen compounds, is naturally converted into nutrients (nitrate) by nitrifying bacteria and used by plants.[9] In this process, plants absorb soluble nutrients for their anabolism and growth, acting as a biological filter[4,10] so that fish production can take place in the same water,[11] cleansed of potentially harmful compounds, such as ammonia and nitrites.[9] Aquaponic systems are considered sustainable agricultural production systems because they reduce land use, water losses and effluent discharges, at the same time as recycling, on average, 98% of the water, and maximizing plant and fish production.[10] Compared with traditional aquaculture, which accounts for almost half of the fish supply for human consumption worldwide,[12] aquaponics can lead to the production of similar amounts of biomass, but only spending a small percentage of the freshwater used in aquaculture.[13] Therefore, aquaponics can benefit the aquaculture operation by producing fish, at the same time as avoiding wastewater discharges and maintaining water quality.[11,14] Hence, because of the potential offered by aquaponics to food production and its sustainability, it is of great interest to conduct research on the implementation and optimization of more of this type of integrated multitrophic systems.

In the present study, the development and control of a novel nutrient-efficient deep-water culture (DWC) aquaponics is described. Fish feed is the major source of nutrients/nitrogen (> 99%) in aquaponics, accounting for up to 70% of the production costs.[9] After being ingested and metabolized, nitrogen is mainly excreted by fish (> 70%) in the form of ammonia (NH_3), and higher fish densities lead to more NH_3 excretion.[9,15,16] Although aquaponic systems are well known by their nitrogen utilization efficiencies (NUE), NUE aquaponics can still be further improved by reducing fish density and NH_3 volatilization and by increasing ammonium-nitrate conversion by nitrifying bacteria. Hence, an efficient aquaponic operation should present high yields of plant and fish biomass with low amount of nitrogen loss. NH_3 volatilization can be reduced by avoiding alkalinity and maintaining a low water pH (~6), to improve NH_3 to NH_4^+ protonation (pKa = 9.3).[14] Thus, we have implemented a low fish density DWC catfish-based aquaponics, supplemented at suboptimal levels (0.5% feed per body weight), for growing four cultivars of fast-growing leafy vegetables: two cultivars of lamb's lettuce (*Valerianella locusta* var. Favor and *Valerianella locusta* var. de Hollande) and two cultivars of arugula (*Eruca vesicaria* var. sativa and *Eruca sativa*). The African catfish (*Clarias gariepinus*) is one of the most farmed fish species because of its high-quality flesh and nutritive value. This omnivorous fish species presents fast growth and is considerably tolerant to environmental stress.[17] Catfish–pumpkin and catfish–spinach aquaponic systems have already been implemented with success.[10,18] Lamb's lettuce (a baby leaf vegetable) and arugula (a cruciferous vegetable) are vegetables of the *Valerianaceae* and *Brassicaceae* families, respectively, being well known as a source of vitamins, minerals and bioactive compounds, such as polyphenols and antioxidants.[19,20] Previous studies have reported the production of arugula and lamb's lettuce in hydroponic systems, as a way of investigating bioaccumulation and biofortification.[21,22] Yet, productions of lamb's lettuce and arugula in aquaponics have never been reported. Thus, the present study aimed to: (i) implement a novel nutrient-efficient catfish-lamb's lettuce and catfish-arugula DWC aquaponics system working at low fish density and suboptimal feed levels; (ii) determine the quality levels of the arugula and lamb's lettuce produced, by monitoring morphological, physical and chemical parameters; (iii) evaluate the influence on plant growth of two light exposure intensities (direct sunlight *vs.* shadow); and (iv) investigate the toxicity imposed by the plant material to human model cells (intestinal Caco-2 cells).

MATERIALS AND METHODS

Aquaponics system

Experimental setup

The study was conducted in a greenhouse on the premises of the Polytechnic of Leiria, city of Leiria (latitude 39°44′37″ N, longitude 8°48′25″ W and 33 m above sea level), in a region characterized by a Mediterranean climate (Köppen-Geiger classification), with moderate average temperatures below 22 °C (~22 °C day, ~10 °C night), rainy winters and dry summers. The setup and dimensions of the catfish-arugula/lamb's lettuce aquaponics system are shown in Table 1 (see also Supporting information, Fig. S1). The aquaponics system comprised a fish-rearing tank; a drum filter

Table 1. Dimensions of the aquaponics system

System components	Dimension
Fish tank	3000 L
Biological filter	300 L
DWC unit	2250 L
Sump	500 L
Truncated-conical sedimentation filter	150 L
Water volume in the system	6450 L
Submersible water pump	10 000 L h^{-1}
System land area occupied	150 m^2

Abbreviation: DWC, deep-water culture.

linked to a sedimentation tank (to remove suspended solids from the aquaculture effluent); a biofilter for nitrification; a hydroponic tank for plant production (hydroponic DWC unit); and a sump tank for water management and recirculation back to the fish tank (see Supporting information). The fish tank capacity was of 3.4 m^3 with 2.0 m in diameter and a water depth of 1.2 m. The DWC hydroponic unit consisted of a rectangular bed (500 × 150 × 40 cm) with eight polystyrene foam rafts (150 × 60 × 4 cm), placed on the water surface. The aquaponics system was initially filled with tap water. Liquid volume in the assembled unit under aeration was 6450 L. The aquaculture effluent flowed into the aerated biofilter filled with plastic bioballs, size 25 × 12 mm (Small Boss MBBR K1 Biofilter, Zhejiang, China) and then to the hydroponic unit. Water recirculated through each unit and no water discharges occurred over the experimental period. No extra lighting, heating or cooling systems were used.

Fish and plants

The fish tank contained 12 African catfish (*C. gariepinus*), varying in length 58.3 ± 5.2 cm (body weight: 1.65 ± 0.28 kg) and at a low fish culture density of 5.8 kg m^{-3}. Fish were obtained from Fleuren & Nooijen BV (Nederweert, The Netherlands). Fish were fed twice a day at 100 g day^{-1} (suboptimal levels of 0.5% feed per body weight),[12] with commercial feed (Tilapia grower 2; Aquasoja, São João de Ovar, Portugal), comprising 37.9% protein, 10.2% lipids, 8.8% ash, 4% fibers, 1.6% calcium, 1.4% phosphor and 0.2% sodium, supplemented with vitamins. No fish disease or mortality was observed during the experiment. Seeds of lamb's lettuce (*Valerianella locusta* var. Favor and *Valerianella locusta* var. de Hollande) and arugula (*E. vesicaria* var. sativa and *E. sativa*) were obtained from commercial companies (Germisem Sementes, Oliveira do Hospital, Portugal; Flora Lusitana, Cantanhede, Portugal). Seeds were sown in a styrofoam nursery bed (October 2019). After 4 weeks, seedlings were randomly chosen from the nursery bed and transplanted into the aquaponic plant tank at a distance of 17 cm between plants, in plastic net pots (2 cm height) filled with expanded clay pebbles. Eight treatments were evaluated in this study, corresponding to a 4 × 2 factorial design, consisting of 34 plants of each of the four cultivars as a first factor, submitted to two different light intensities inside the greenhouse: direct sunlight and shadow (manually regulated via a shadowing screen), as a second factor, with half of the plants of each cultivar receiving direct sunlight. Photosynthetic photon flux density (PPFD) and illuminance were measured daily (12:00 am) by making use of a LI-250A light meter (LI-COR Biosciences, Bad Homburg vor der Höhe, Germany) and a PCE-174 Datalogging light meter (PCE Instruments, Meschede, Germany), respectively. PPFD values were of 369.6 ± 289.5 μmol m^{-2} s^{-1} under sunlight and 118.5 ± 66.4 μmol m^{-2} s^{-1} in the shadow, while illuminance was of 20 044.5 ± 15 123.4 lm m^{-2} (sunlight) and 4991.4 ± 2653.4 lm m^{-2} (shadow). The photoperiod (natural daylight) was 10 h on averag (07.00 h to 17.00 h).

Air and water quality parameters

Greenhouse air temperature and relative humidity were measured hourly (24 measurements per day) with a LogTag HAXO-8 (LogTag, Aukland, New Zealand). Tank water temperatures were measured daily, along with pH, dissolved oxygen (mg L^{-1}), electric conductivity (mS cm^{-1}), oxidation reduction potential (mV) and total dissolved solids (mg L^{-1}), by making use of a multiparameter probe (Edge HI2030/HI763100; Hanna Instruments, Villafranca Padovana, Italy). Additional water parameters of the DWC unit were measured weekly, such as ammonium, nitrite, nitrate, phosphate, iron and potassium concentrations (mg L^{-1}). Water samples were collected 12 h after feeding, to allow fish digestion, excretion and wastewater processing by the biofilter. Sample electrical conductivity (EC), dissolved oxygen (DO), oxidation reduction potentials (ORP) and pH were measured directly. Nitrate was determined by the brucine method.[23] Nitrite was determined according to the SMEWW 4500-NO_2^-—B method.[24] Ammonia nitrogen (NH_3—N) was determined according to ISO 7150-1:1984. Dissolved phosphorus (phosphate) was determined according to SMEWW-P E.[24] Contents in iron and potassium were determined by flame atomic absorption spectroscopy (SpectrAA 55B; Varian Inc., Palo Alto, CA, USA), according to the SMEWW 3111B method,[24] with iron (248.3 nm) (Heraeus, Hanua, Germany) and potassium (766.5 nm) (Agilent, Santa Clara, CA, USA) hollow cathode lamps, respectively. For colorimetric determinations, water samples were previously filtered through cellulose nitrate Sartopore 0.45-μm pore diameter membranes (Sartorius, Goettingen, Germany) to remove suspended solids. Total coliforms were assayed routinely (ISO 9308-1). *Escherichia coli* was never detected, and total coliforms were always within acceptable limits (< 10^4 UFC g^{-1}).

Morphological measurements

Plant survival, health status and nutrition were monitored weekly by inspecting growth and overall look. Plants were morphologically analyzed according to several parameters during five consecutive weeks for each cultivar of arugula and of lamb's lettuce in a period of 5–7 weeks (October to December). The four cultivars, submitted to two different light intensities, were evaluated in terms of height (cm), health status (weak/strong), color (leaf greenness: low/high), foliage diameter (cm), leaf number, biggest leaf length (cm) and root length (cm). Measurements were performed with a measuring tape. The number of leaves was counted considering all observable leaves with two or more centimeters. After the 5-week growth period, plants were harvested and immediately weighed, with and without roots with a 0.0001 g precision scale (262SMA-FR; Precisa Gravimetrics, Dietikon, Switzerland) and subsequently submitted to chemical analysis.

Analytical reagents

All chemicals were of analytical grade or higher. The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC, Porton Down, UK). Fetal bovine serum was acquired from ThermoFisher Scientific Inc. (Walthem, MA, USA). Dulbecco's modified Eagle's medium high glucose (DMEM), non-essential amino acids, penicillin (100 U mL^{-1}), streptomycin (10 mg mL^{-1}) and the trypsin–ethylenediaminetetraacetic acid solution were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Isotope-labelled compounds were purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA, USA). Ultrapure Milli-Q Type 1 (Millipore, Burlington, MA, USA) water was used in all analytical determinations.

Chemical measurements

Antioxidant capacity

Antioxidant capacity (AC) of plant extracts was evaluated by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (DPPH) assays, as described previously.[25] Extracts were prepared by placing the plant material in 70% (v/v) acetone at a 1:10 ratio and stirring for 2 h on a magnetic stirrer at 5000 x g and 20 °C. Samples were filtered through qualitative paper filters (~22 μm) and centrifuged for 20 min at 4000 × *g* (Labofuge 200; Heraeus). Trolox was used as the standard compound for the calibration curves. ABTS and DPPH inhibition activities (%) were calculated as: % inhibition = [(Control Abs − Sample Abs)/Control Abs] × 100. Antioxidant activities were expressed in milligrams of Trolox equivalent antioxidant capacity (TEAC) per gram of fresh weight of leaf material (mg TEAC g^{-1}). All determinations were carried out in quadruplicate. Spectroscopic measurements were performed on a Cary UV-visible spectrophotometer (Varian Inc.).

Total phenolic content

Plant extracts in 70% (v/v) acetone (1:10 ratio) were tested for phenolic compounds by the Folin–Ciocalteau assay, as described previously.[25] Gallic acid was used as the standard compound for the calibration curve. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight (mg GAE g^{-1}). To attest for the presence of phenolic groups in the plant's extracts, NMR experiments were also carried out. NMR samples were prepared by adding frieze dried (Kinetics, Eschau Hobbach, Germany) material to deuterated acetone-d_6 70% (v/v) and centrifugation for 20 min at 4000 × *g* (Labofuge 200; Heraeus). NMR spectra were obtained on a Bruker Avance III 400 spectrometer (Bruker, Wissembourg, France) operating at a ^{1}H frequency of 400.133 MHz, at 25 °C. One-dimensional ^{1}H NMR spectra were obtained with 16 k complex points, 512 scans, a spectral width of 6402 Hz and a recycle delay of 2 s. Processing was performed with Topspin, version 4.0 (Bruker) and MestReNova, version 9.1 (Mestrelab, Santiago de Compostela, Spain). Chemical shifts were reported in ppm, relative to the methyl group $-CH_3$ ^{1}H ($\delta = 2.05$ ppm) resonance of acetone-d_6, used as external reference.

Caco-2 cell viability tests

Plant material was tested for cell viability with the human colon adenocarcinoma Caco-2 cell line, as described previously.[25] Leaves were freeze-dried, macerated and added to the DMEM medium. Cells were incubated with increasing concentrations of plant material for 24 h. Then, cells were washed with phosphate buffer saline and incubated with 1.20 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for 4 h, at 37 °C. Finally, the medium was discarded, and the formazan precipitates dissolved with dimethyl sulfoxide. Absorbance was measured at 570 nm (Synergy HT Microplate Reader; BioTek Instruments, Winooski, VT, USA). Cells incubated with DMEM were considered as controls (100% cell viability). Each experiment was performed in quadruplicate.

Statistical analysis

Statistical analyses were performed with SPSS, version 26 (IBM Corp., Armonk, NY, USA). Categorical variables were analyzed through frequency analysis, contingency tables and association measures. Quantitative variables were analyzed via the mean ± SD. Hypothesis tests, including multivariate analysis of variance (MANOVA), were applied to assess plant growth and the influence of the two independent factors: *cultivar* and *light intensity*. MANOVA analysis assumed data normality (checked by the Kolmogorov–Smirnov test) for dependent variables within factor groups, as well as homogeneity of the covariance matrix (checked by the Box's *M* test). MANOVA statistic tests (Wilks' lambda, the Pillai's trace, the Hotelling's trace and the Roy's largest root test) analyzed the combined effect of the two independent factors, 'cultivar' and 'light intensity', on the average values of each of the six measurements collected for each variable [plant height (cm), foliage diameter (cm), leaf number, biggest leaf length (cm) and root length (cm)]. Multiple linear regressions were also applied to produce parsimonious mode to predict variations in plant weight as a function of the morphological characteristics measured at harvest. Mann–Whitney *U*-tests were conducted to estimate contents in antioxidants and polyphenols.

RESULTS AND DISCUSSION

Performance of the aquaponics system

Greenhouse air temperature ranged from 5.0 °C (night) to 37 °C (day), with mean temperature of 16.3 ± 5.9 °C and mean relative humidity of 37.1 ± 9.0% (see Supporting information, Fig. S2). Table 2 presents minimum, maximum and mean values of temperature, dissolved oxygen (DO), electric conductivity (EC), oxidation reduction potentials (ORP) and total dissolved solids (TDS) in

Table 2. Water quality parameters of the aquaponics system

Parameter		Minimum	Maximum	Mean ± SD
Water temperature (°C)	Fish tank	17.3	22.1	20.3 ± 0.97
	DWC	17.2	22.0	20.2 ± 0.99
DO (mg L^{-1})	Fish tank	4.4	9.1	7.1 ± 1.00
	DWC	2.6	7.4	5.3 ± 1.02
pH	Fish tank	5.1	6.8	6.1 ± 0.36
	DWC	4.9	7.2	6.2 ± 0.36
EC (μS cm^{-1})	Fish tank	185	369	270 ± 29.4
	DWC	194	372	273 ± 29.8
TDS (mg L^{-1})	Fish tank	108	185	145 ± 12.9
	DWC	114	202	147 ± 13.1
ORP (mV)	Fish tank	87	330	227 ± 61
	DWC	103	351	227 ± 56

Note: Values are the mean ± SD.
Abbreviations: DO, dissolved oxygen; EC, electric conductivity; TDS, total dissolved solids; ORP, oxidation reduction potential, DWC, Deep-water-culture.

the fish rear-tank and DWC unit, during the experiment. Throughout plant growth (see Supporting information, Fig. S3), water temperature varied from 17.3/17.2 °C (morning) to 22.1/22.0 °C (evening) in the fish tank and DWC unit, respectively, with average values of 20.3/20.2 ± 1.0 °C, which are adequate to maintain fish health and wellbeing.[26] These small variations in temperature of the aquaponics system (~5 °C amplitude) showed the relevance of the greenhouse in preserving heat. Additionally, fish tank and DWC water temperature were almost identical, highlighting the thermal efficiency of the aquaponics system. Regarding dissolved oxygen (DO), mean values of 7.10 ± 1.0 mg L^{-1} were detected in the water of the fish rear-tank, with minimal and maximal values of 4.4 and 9.1 mg L^{-1}, correspondingly (Table 2). DO mean values ranging from 5.0 to 8.0 mg L^{-1} are considered optimal for the development of aquatic life in any culture system, even if the catfish (which has an accessory respiratory organ) can tolerate DO values as low as 1.0 mg L^{-1}.[27] Subsequently, lower DO mean values were detected in the DWC unit (5.30 ± 1.02 mg L^{-1}) as a result of oxygen consumption by fish respiration and bacterial aerobic metabolism. Concerning pH, EC, TDS and ORP (Table 2), mean values were similar between the fish tank and the DWC unit. Water pH in the aquaponics system was maintained at optimal values (6.2 ± 0.4) to avoid nitrogen loss by NH_3 volatilization and to guarantee adequate availability and uptake by the radicle system.[28,29] EC remained at low values (273 ± 29.8 $\mu\text{S cm}^{-1}$), adequate for leafy vegetables, because excessive EC values are known to induce salinity stress, and force plants to increase the activity of antioxidant enzymes, not only as a way to adapt, but also compromising plant growth and tissue quality.[30] Likewise, as a parameter related to EC, total dissolved solids (TDS) were kept at adequately low values (< 200 mg L^{-1}).[31] Mean values of ORP also remained within a suitable range (200–400 mV). ORPs were sufficiently high to promote minimal/basal bacterial growth essential for nitrogen conversion, but not too excessive, which otherwise would totally inhibit bacterial and plant growth. ORP values were also consistent with the pH values measured, as well as with the DO available to fish and plant roots.

Nutrient concentrations in the DWC water are shown in Table 3. In general, as expected because of the fish feed, nutrient levels gradually increased over time, specifically, from 78 to 90 mg L^{-1} for nitrate, from 8.1 to 8.9 mg L^{-1} for phosphates and from 3.11 to 5.87 mg L^{-1} for potassium. Gradual NO_3^-–N accumulation indicates that the N release rate derived from fish feed (administrated at suboptimal levels, 0.5% feed per body weight), and from ammonium and nitrite conversion by nitrifying bacteria, outpaced the NO_3^-–N uptake by the roots (needed for plant growth, ~100 $\text{mgNO}_3^-\ \text{L}^{-1}$).[12] Water contents in toxic ions such as ammonium (NH_4^+–N) and nitrite (NO_2^-–N) were always low throughout the experiment (Table 3). Ammonium (< 0.2 mg L^{-1}) and nitrite (< 0.068 mg L^{-1}) were kept at adequate levels for fish production (NH_4^+–N < 0.2 mg L^{-1} and NO_2^-–N < 1.1 mg L^{-1}),[9] given their conversion to NO_3^-–N by nitrifying bacteria. Iron levels were also low (< 0.1 mg L^{-1}) and appropriate for aquaponics.[31] As reported previously for other aquaponic systems, a sharp initial decrease (~50%) in phosphates (PO_4^{3-}–P) was also observed in the present study after week 1 (Table 3). Thus, as a result of the gradual raise in calcium and phosphates resulting from fish feed and metabolism, after 1 week of functioning, a sudden drop in phosphate was observed because of precipitation.[28] Additionally, pH values also decreased gradually (~pH 5.0), as expected,[14] along with a simultaneous raise in nitrate (Table 3). Beyond the nitrification process (i.e. that consumes alkalinity), ammonium and CO_2 production from fish metabolism and oxidation of organic matter also contribute to pH lowering.[9] Nonetheless, after proper pH adjustment (with a saturated solution of potassium hydroxide) (see Supporting information, Fig. S3C, week 2), these parameters reverted to adequate values, with higher ORPs (~300 mV) (see Supporting information, Fig. S3F, week 2) ideal for the growth of the nitrifying bacteria and of the leafy vegetables under production. Total fish feed applied (3500 g) led to a plant biomass of 206.9 g (125.7 g of lamb's lettuce and of 81.2 g of arugula) and a fish biomass of approximately 20 000 g.

Morphological characteristics of the plants

Plant specimens were examined daily and measured weekly in terms of plant height (cm), foliage diameter (cm), leaf number, biggest leaf length (cm) and root length (cm). Figure 1 plots the mean values for the different cultivars, grown under two different light levels (direct sunlight–PPFD ~400 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$/shadow–PPFD ~120 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$). By the end of the experiment, plants reached the final average height of 7.1 ± 1.9 cm for lamb's lettuce and 7.2 ± 2.4 cm for arugula. At the end of the experiment (see Supporting information, Fig. S4), plants were harvested and inspected in terms of *health status* (weak/strong) and *greenness* (low/high). The categorical variable *greenness* was measured according to: 'low' (plants discolored or with pale green leaves) and 'high' (plants with medium or high levels of greenness). *Health status* was measured according to: 'weak' (symptomatic plants exhibiting typical signs of disease, such as yellowing or stiffness loss) and 'strong' (asymptomatic plants or plants with few or no signs of disease).

Cultivars of lamb's lettuce grown at two light intensities

Average values of 'biggest leaf length' and 'foliage diameter' were significantly different among cultivars ($P < 0.01$), with higher values for var. de Hollande (Fig. 1), reflecting the original differences between cultivars. In turn, the effect of the factor 'light intensity' on the average biggest leaf length was not significant

Table 3. Chemical parameters of the water of the deep-water culture (DWC), registered weekly

Parameter	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Ammonium (mg NH_4^+–N L^{-1})	< 0.06	0.22	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Nitrates (mg NO_3^-–N L^{-1})	78	75	85	77	73	90	99	90
Nitrites (mg NO_2^-–N L^{-1})	0.068	0.050	0.035	0.021	< 0.02	0.032	0.028	0.062
Phosphates (mg PO_4^{3-}–P L^{-1})	8.1	3.6	9.2	8.6	9.5	8.7	9.3	8.9
Iron (mg Fe L^{-1})	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Potassium (mg K L^{-1})	3.11	2.99	6.37	6.56	5.45	6.95	5.21	5.87

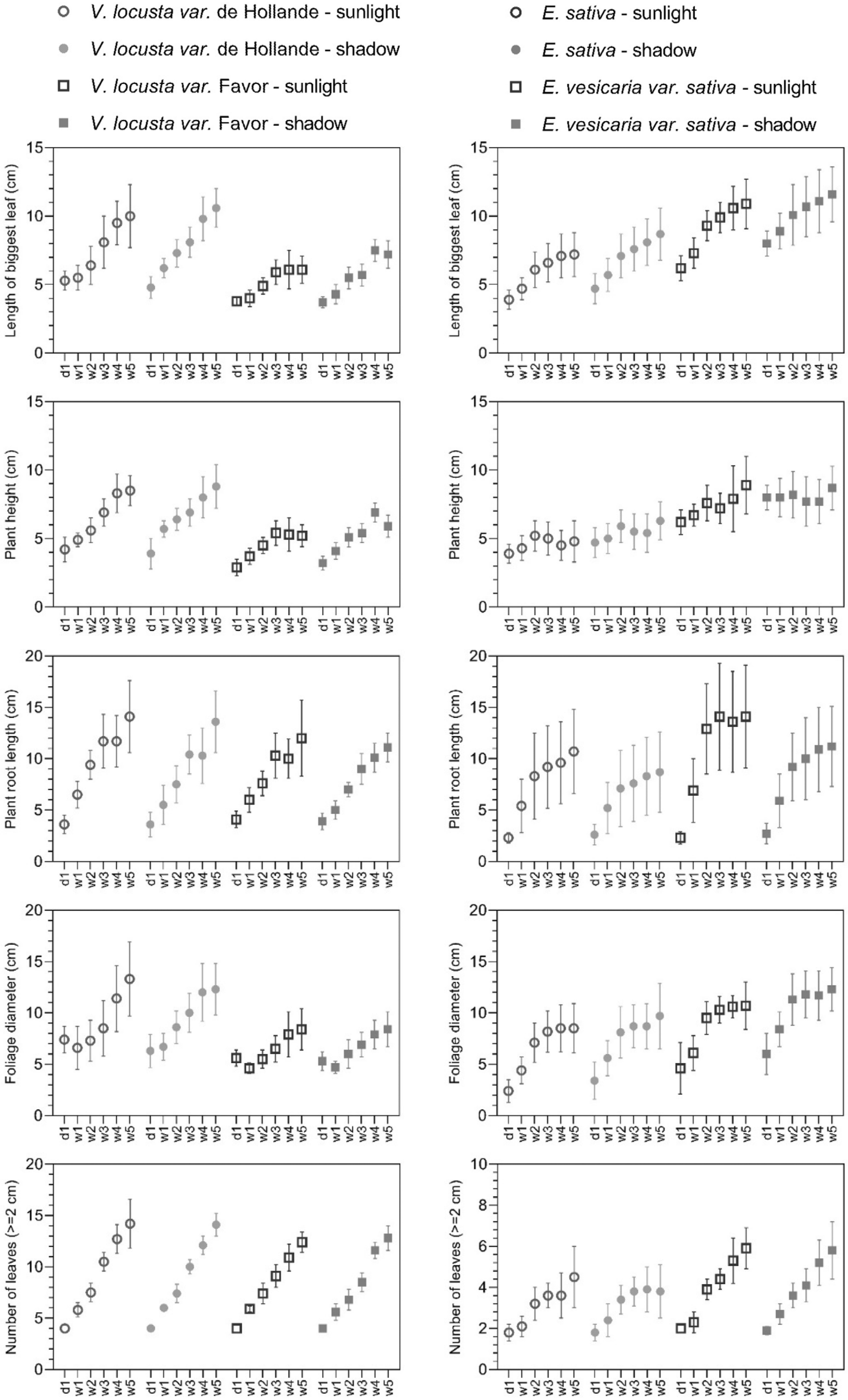

Figure 1. Mean values of the morphological parameters for lamb's lettuce (left) and arugula (right): biggest leaf length; plant height; root length; foliage diameter and number of leaves (≥ 2 cm), collected during the 5-week growth period.

($P > 0.01$), but showed slightly higher values for plants grown in the shadow (~120 μmol m^{-2} s^{-1}). The interaction effects between cultivar and light intensity for the three variables 'biggest leaf length', 'root length' and 'foliage diameter' were found not to be significant ($P > 0.01$). However, both factors were separately shown to have a significant effect on at least one of the weekly variables ($P \approx 0$). Regarding the variable 'root length', no significant differences were detected between cultivars, although, from week 1 to week 3, a significant influence was found ($P < 0.01$) between light intensity and root length, with higher values for plants exposed to direct sunlight (PPFD ~400 μmol m^{-2} s^{-1}). Conversely, the interaction between cultivar and light intensity (Fig. 1) on the average values of each of the six measurements was found to be significant, by all MANOVA statistical tests ($P < 0.01$). Moreover, for the variable 'leaf number', a two-way ANOVA was used to test whether the means of the six measurements collected were influenced by the effect of the two factors under study. The results showed no significant interaction between cultivar and light exposure over time (1% significance). Nonetheless, after reaching an intermediate growth stage (week 3), the average 'leaf number' was significantly different between cultivars ($P < 0.05$), with higher values for var. de Hollande.

After the 5 week growth period, all plant specimens were harvested, weighed and examined in terms of 'greenness' and 'health status' (Table 4). The 67 plant specimens of lamb's lettuce weighed (without roots) a total of 107.6355 g (1.6065 ± 0.8576 g per plant), whereas all roots weighed 18.0936 g (root/shoot fresh biomass of 16.8%). These results enabled to produce a parsimonious model of weight as a function of 'foliage diameter' and 'leaf number', which explained 87.3% of the variation in plant weight. Hence, for each 1-cm increase in foliage diameter, the estimated increase in plant weight would be of about 0.206 g, whereas, for each increase in one leaf, and the estimated increase in plant weight would be of about 0.084 g. At harvest, the great majority of plants from both cultivars, submitted to both light intensities, showed high levels of greenness and health status, with no signs of disease. Even so, var. de Hollande showed a higher percentage of green and healthy plants (97%) compared to var. Favor (88%).

The results indicate that, although both cultivars were shown to adapt well to the aquaponics system implemented, at both light intensities, var. de Hollande adapted even better than var. Favor, as corroborated by the higher average values of the quantitative variables. Lamb's lettuce specimens were able to present higher number of leaves (> 14 leaves) and root (~0.27 g) and shoot (~1.61 g) average fresh weights close to other lamb's lettuce plants (*V. locusta*) grown hydroponically.[21,32] Hence, the two Lamb's lettuce cultivars were able to grow and prosper (with high greenness and healthy status) at the low nitrogen conditions (suboptimal levels of 0.5% feed per body weight and low fish density 5.8 kg m^{-3}) implemented here. These conditions avoided the use of high feed inputs that would lead to the accumulation of unused nitrogen in the fish tank, increasing the amount of ammonium (highly toxic for fish, even at low concentrations) released by fish feces and food decomposition.[33] Even if nitrogen/nitrate-imbalance in plants can lead to smaller specimens and lower yields (given its relevance for plant growth and crop production),[9] when ingested through food consumption, nitrates are converted to nitrites by salivary bacteria or to other toxic compounds (e.g. nitrosamines–carcinogenic compounds) extremely harmful to human health, especially to some population groups (vegetarians and babies).[34] Thus, the World Health Organization has defined 3.7 mg.kg^{-1} per body weight,[35] as the acceptable daily intake for nitrate (NO_3–) and the European Commission has defined maximum nitrate limits for leafy vegetables (Regulations EC/N-1881/2006 and EC/N-1258/2011): lettuce (3–5 g kg^{-1} fresh weight), lettuce type 'Iceberg' (2–2.5 g kg^{-1} fresh weight) and arugula/rocket salad (6–7 g kg^{-1} fresh weight) (with arugula being a well-known nitrate hyper-accumulator species). Therefore, plant nitrate levels can be modulated using different cultivation strategies and maintained at minimal levels to guarantee food safety.[36,37] Moreover, given the crucial role of light in driving phytochemical biosynthesis and photosynthetic activity, both lamb's lettuce cultivars exposed to the highest light intensity tested in the present study (~400 μmol m^{-2} s^{-1}), showed better morphological and quality parameters than at low intensity (~120 μmol m^{-2} s^{-1}), as previously seen for other leafy vegetables. Melody spinach grown under light intensity levels of 200 to 335 μmol m^{-2} s^{-1} was able to accumulate higher levels of

Table 4. Greenness and health status of the plant specimens at harvest, grown at two light intensities

Cultivar	Light intensity	Greenness High	Greenness Low	Health status Strong	Health status Weak	Total
Lamb's lettuce[a]						
Valerianella locusta var. Favor		29	4	29	4	33[c]
	Direct sunlight	15	1	15	1	
	Shadow	14	3	14	3	
Valerianella locusta var. de Hollande		33	1	33	1	34
	Direct sunlight	17	0	17	0	
	Shadow	16	1	16	1	
Arugula[b]						
Eruca sativa		9	24	4	29	33[c]
	Direct sunlight	1	16	1	16	
	Shadow	8	8	3	13	
Eruca vesicaria var. sativa		6	28	2	32	34
	Direct sunlight	0	17	0	17	
	Shadow	6	11	2	15	

[a] Harvested the 11 December 2019.
[b] Harvested the 27 November 2019.
[c] One specimen perished.

macronutrients and pigments than at lower light intensity (125 µmol m^{-2} s^{-1}).[38] Several vegetables (soil cultured) such as green lettuce, lamb's lettuce and arugula/rocket were also able to show higher nutritional value in terms of protein, K, Ca and Mg contents when harvested at light intensities ranging from 200 to 400 µmol m^{-2} s^{-1}.[20]

Cultivars of arugula grown at two light intensities

Mean values of biggest leaf length and plant height were found to be significantly different among cultivars ($P < 0.01$), with higher values for the cultivar *E. vesicaria* var. sativa (Fig. 1), substantiating the original differences between cultivars. As performed for lamb's lettuce, a MANOVA analysis was used to analyze the combined effect of the independent factors tested (*cultivar* and *light intensity*). No significant interactions were found between factors. Nonetheless, the results led to conclude that both factors separately had a significant ($P \approx 0$) effect on at least one of the weekly variables measured. Additionally, in what is regarded as the effect of direct sunlight/shading exposure on the average biggest leaf length and plant height, the factor was significant ($P < 0.01$), although only at an early stage of growth (< 3 weeks), with slightly higher values for plants submitted to a lower light intensity. Plant and leaf sizes result from a balance between the need to maximize energy uptake, at the same time as minimizing stress caused by environmental factors, such as temperature, light quality/intensity or ambient humidity.[39,40] Hence, plants in the present study growing in the shade were not only more protected from reaching higher temperatures, but also able to adapt their height and leaf growth, to maximize light quality and intensity.[39]

Regarding the variables 'root length' and 'foliage diameter', the MANOVA analysis showed that no significant ($P < 0.01$) interactions were detected between the two factors 'cultivar' and 'light intensity' on the average values of each of the six measurements associated to both variables. Nonetheless, even if the factor cultivar did not show a significant effect ($P > 0.05$) on the weekly measurements of the variable root length, the factor light intensity showed statistical significance ($P < 0.05$). In addition, both factors separately had a significant effect ($P < 0.001$) on at least one of the weekly measurements, namely foliage diameter. Moreover, from week 2 onward, the effect of the factor light intensity on the average root length of the plants was shown to be significant ($P < 0.01$), with slightly higher values for plants exposed to direct sunlight. Concerning foliage diameter, the average value measured over time was significantly different between cultivars ($P < 0.01$), with higher values shown by *E. vesicaria* var. sativa, as well as significantly different between light intensities ($P < 0.01$), with higher values for plants growing in the shade. Hence, environmental factors played a role and can explain the observed differences. Plants shielded from direct sunlight (PPFD ~120 µmol m^{-2} s^{-1}) were able to thermoregulate more efficiently and grow in height and foliage diameter.[39] Conversely, plants submitted to direct sunlight (PPFD ~400 µmol m^{-2} s^{-1}), showed smaller foliage diameter (to diminish evaporation–transpiration processes), but presented longer roots (with *E. vesicaria* var. sativa roots reaching 14 cm at PPFDs of 400 µmol m^{-2} s^{-1} compared to 11 cm at PPFDs of 120 µmol m^{-2} s^{-1}) (Fig. 1), to maximize water intake.[39]

The results regarding leaf number were found to be similar to those obtained for lamb's lettuce, when using a two-way ANOVA. Likewise, the interaction between cultivar and light intensity over time was not significant. However, statistical significance was observed for the average leaf number measured over time for the two different cultivars, with higher values for *E. vesicaria* var. sativa ($P < 0.05$).

After a 5-week period, arugula plants were harvested, weighed and examined in terms of 'greenness' and 'health status' (Table 4). The 67 specimens weighed (without roots) a total of 79.0537 g (1.1799 ± 0.7125 g per plant), whereas all roots weighed 2.1706 g (root/shoot fresh biomass of 2.8%). These results allowed to produce a parsimonious model representing weight as a function of biggest leaf length and leaf number, which explained 80.5% of variation in plant weight. For each 1-cm increase in the biggest leaf length, the estimated increase in plant weight would be about 0.197 g, whereas, for every new leaf, the estimated increase in plant weight would be around 0.156 g. Both cultivars of arugula showed a considerable number of plant specimens (> 70%) with some degree of yellowing and pale leaves, as well as some loss of stiffness and signs of disease (Table 4). Even so, some plants were still able to maintain high levels of greenness and health status throughout the experiment, especially the specimens submitted to lower light intensity. Additionally, the chi-squared test of independence also led to the conclusion that significant independence ($P = 0.345$) existed between the variable's cultivar and greenness, whereas significant dependence ($P \approx 0$) existed between light intensity and greenness, given the lower tolerance of *E. vesicaria* to higher temperatures and higher intensity light exposure.[30] Even so, these arugula specimens grown aquaponically presented plant heights (~10 cm) and number of leaves (~6 leaves) similar to soil-grown plants, developed under water restriction.[41] As opposed to lamb's lettuce, none of the cultivars of arugula showed full adaptation to the aquaponics system, at any of the light intensities tested. Arugula cultivars are known to be more sensitive to temperature effects and to grow better at temperatures ranging from 10 to 20 °C[30] compared to lamb's lettuce cultivars that are more tolerant to higher temperatures (~35 °C).[42] Thus, heat stress can severely limit crop productivity. High temperatures (> 40 °C) can retard plant germination, growth, regrowth and survival of arugula/rocket plants.[43] Imposition of high/low temperature stress leads to the expression of heat shock proteins (HSPs) and transcription factors. HSPs act as molecular chaperones by preventing protein denaturation, misfolding and aggregation and by controlling signal transduction during heat stress.[44] In turn, transcription factors such as MYC2 and MYB28 have been associated with the control of abiotic challenges, by shaping ammonium stress and iron metabolism,[45] as well as by promoting the biosynthesis of glucosinolates, at the cost of plant growth and development,[43] as seen here for *E. vesicaria* var. sativa. In addition, heat stress also affects pigment production (chlorophyll a), impairing the photosynthetic machinery,[46] which explains the loss of greenness and some degree of yellowing and pale leaves presented by some of the arugula specimens grown under direct sunlight (PPFD ~400 µmol m^{-2} s^{-1}).

Climate change towards higher temperatures, either occurring permanently or temporarily, is a major environmental problem that directly affects agricultural production and food security. Thus, future aquaponic systems for growing arugula cultivars must consider the use of energy-efficient acclimatized greenhouses. When comparing cultivars, *E. sativa* were shown to adapt slightly better than *E. vesicaria* var. sativa, as suggested by the levels of greenness and health status presented by the plants, especially those growing in the shade (~120 µmol m^{-2} s^{-1}).

Chemical characteristics of the plants

Cruciferous vegetables are known to be rich in bioactive compounds, such as antioxidants and polyphenols, with recognized nutraceutical properties.[42,47,48] These additional/secondary metabolites work as scavengers of reactive oxygen species (ROS), as an adaptive response to stress conditions caused by drought, salinity, extreme temperatures, lack of light intensity or intense sunlight.[49-52] Thus, the number of antioxidants produced by the plant generally correlates with the environmental stress suffered during growth.[53-55] Hence, TPCs and antioxidant capacities were determined for the leaf extracts of the four cultivars, submitted to the two different light intensities (direct sunlight PPFD ~400 μmol m^{-2} s^{-1}/shadow PPFD ~120 μmol m^{-2} s^{-1}) (Table 5).

Concerning arugula, results are within the range of TPCs previously reported (~ 2.0 mg GAE g^{-1} fresh weight).[56] In general, arugula extracts presented slightly higher phenolic contents, at both light intensities tested, than lamb's lettuce extracts. Additionally, both cultivars of arugula showed higher TPCs for the specimens submitted to higher light intensity (direct sunlight) compared to the plants grown under shaded light, as indicated by the 'strong' association of 0.858, reported by the Eta coefficient, and by the significant Mann–Whitney *U*-test ($P < 0.05$). When comparing cultivars, *E. vesicaria* var. sativa showed higher average TPCs, in agreement with the lower levels of greenness and health status presented by the plants at harvest. Regarding the TPCs for lamb's lettuce, no major differences were observed between cultivars at high light intensity (PPFD ~400 μmol m^{-2} s^{-1}). Nonetheless, some differences, although not significant, were observed at low light intensities (PPFD ~120 μmol m^{-2} s^{-1}), with lower TPCs for var. de Hollande (more adapted to the aquaponics system) and higher TPCs for var. Favor (shadow) (Table 5), indicating that, to better adapt to the environmental conditions experienced during growth, plant specimens of var. Favor had to exacerbate the activity of their antioxidant enzymes, as well as the production of non-enzymatic antioxidants such as carotenoids, tocopherols and phenolics.[57]

In addition, 1D ^{1}H NMR spectra were also collected for the acetonic extracts of the four cultivars, submitted to the two light intensities tested (see Supporting information, Fig. S5). The downfield region of the NMR spectra (8.5–6.0 ppm) showed a very similar profile of peaks, both in terms of chemical shifts and intensities, regardless of the cultivar and of the light intensity applied. All spectra showed typical resonances of ortho- (vicinal coupling $^3J \sim 8$ Hz) and meta- (vicinal coupling $^3J \sim 2$ Hz) protons of phenolic moieties present in phenolic compounds, such as flavonoids (e.g. quercetin and kaempferol) and hydroxybenzoic acids (e.g. caffeic, ferulic and chlorogenic acids).[58] Hence, even if all plants produced similar amounts of polyphenols, to overcome some of the abiotic stress felt during growth, *E. vesicaria* var. sativa produced higher amounts of polyphenols compared to *E. sativa*, at both light intensities. These results agree with the morphological quantitative and qualitative measurements described above. The cultivar *E. sativa* was also shown to be better adapted to the aquaponics system, as judged by the slightly higher levels of greenness and health status shown by the plants (Table 4), relative to the lower levels shown by *E. vesicaria* var. sativa.

In turn, concerning the NMR spectra of the two cultivars of lamb's lettuce, a similar pattern of peaks, also assigned to protons of phenolic acids, could be observed in the aromatic region of the spectra (8.5–6.0 ppm). As for arugula, the NMR data correlates well with the amount of TPCs detected by the FC method (Table 5). Nonetheless, weaker signals were observed, indicating that these plants did not need to exacerbate their antioxidant mechanisms to thrive and grow. In addition, differences were found between cultivar and light intensity for lamb's lettuce extracts. The cultivar presenting less intense NMR peaks assigned to protons of phenolic moieties was *V. locusta* var. de Hollande, which is in agreement with lower levels of abiotic stress suffered, as well as with the higher levels of greenness and health status presented by the plants (Table 4), in addition to higher values of biggest leaf length, root length, foliage diameter, leaf number and plant height.

The AC measured for the four cultivars of plants submitted to the two light intensities also agrees with the TPCs obtained. In general, arugula cultivars showed higher ACs compared to lamb's lettuce cultivars (Table 5), especially those submitted to higher light intensities during growth (as supported by the 'reasonable' association described by the Eta coefficient = 0.645, and by the significant Mann–Whitney *U*-test, $P < 0.05$). Additionally, the ACs detected by both methods (ABTS and DPPH assays) are within the range of previous reports[56,59] and directly correlate (Pearson's correlation 0.576) with the results obtained by the FC method and 1D ^{1}H NMR data, indicating that most of the antioxidant capacity detected is a result of the presence of phenolic compounds. With

Table 5. Total phenolic content (TPC) and antioxidant capacity (AC) of leaf acetonic extracts of the four cultivars, grown at two light intensities

	TPC (mg GAE g^{-1})		AC (mg TEAC g^{-1})			
	FC		ABTS		DPPH	
Cultivar	Sunlight	Shadow	Sunlight	Shadow	Sunlight	Shadow
Lamb's lettuce cultivars						
Valerianella locusta var. Favor	1.45 ± 0.07 a	3.70 ± 0.12 a	3.63 ± 0.02 a	4.55 ± 0.02 a	0.20 ± 0.10 a	0.45 ± 0.24 a
Valerianella locusta var. de Hollande	1.49 ± 0.02 b	1.12 ± 0.13 b	4.79 ± 0.02 b	3.41 ± 0.15 b	0.20 ± 0.10 b	0.31 ± 0.18 b
Arugula cultivars						
Eruca sativa	2.80 ± 0.02 c	1.48 ± 0.02 c	4.90 ± 0.02 c	4.21 ± 0.02 c	0.90 ± 0.07 c	0.45 ± 0.16 c
Eruca vesicaria var. sativa	3.62 ± 0.02 d	2.21 ± 0.02 d	4.49 ± 0.02 d	4.12 ± 0.04 d	1.32 ± 0.07 d	0.69 ± 0.25 d

Note: Different lowercase letters indicate significant differences ($P < 0.05$).
Abbreviations: GAE, gallic acid equivalents; TEAC, Trolox equivalents antioxidant capacity; FC, Folin–Ciocalteu assay; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation-based assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical-based assay Values are the mean ± SD of triplicates of three independent samples.

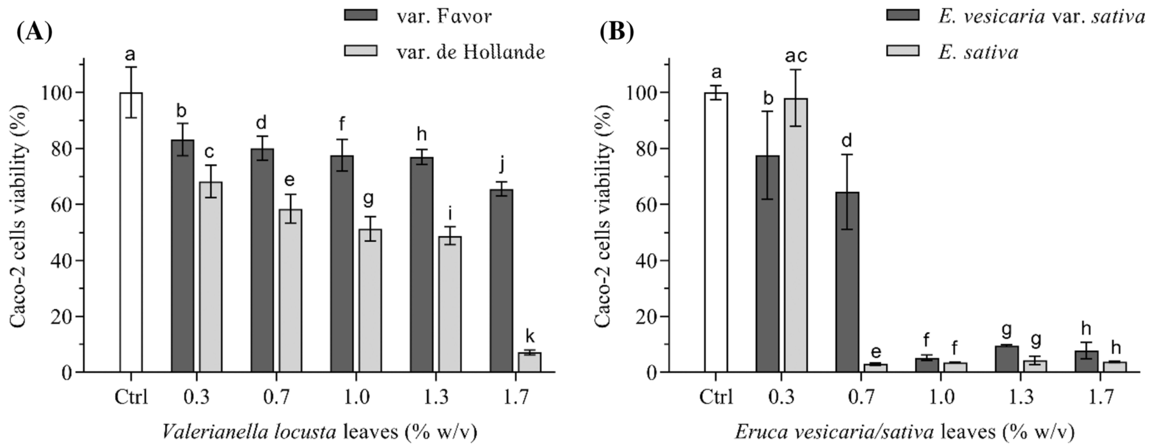

Figure 2. Caco-2 cell viability assayed by the MTT test. Cell viability upon 24-h exposure to increasing concentrations of plant material of the two cultivars of lamb's lettuce *V. locusta* var. Favor and *V. locusta* var. de Hollande (A) and of the two cultivars of arugula *E. vesicaria* var. sativa and *E. sativa* (B). Controls were incubated with DMEM (100% viability). Results are presented as the mean ± SD of triplicates of three independent samples. Different lowercase letters indicate significant differences ($P < 0.05$).

respect to the AC obtained for the extracts of lamb's lettuce (Table 5), a different pattern was observed. The AC detected by the ABTS assay directly correlates with the results obtained by the FC method and NMR data, whereas the DPPH results point towards lower values of AC, which are particularly low for specimens submitted to higher levels of light intensity (as supported by the 'reasonable' association described by the Eta coefficient = 0.458, and by the significant Mann–Whitney *U*-test, $P < 0.05$). Given that the DPPH assay is more efficient in detecting hydrophobic antioxidants,[25,58] the results indicate that most of the AC is related to the presence of more hydrophilic compounds, such as polyphenolic acids. Previous studies have also shown that high intensity artificial LED light (> 600 μmol m^{-2} s^{-1}) was able to induce the production of antioxidants in 40-day-old *E. sativa* plants, at the same time as reducing plant biomass,[60] as was also observed for other vegetables.[61,62] For some leafy vegetables, such as lettuce and spinach, a low light intensity (PPFD 120–180 μmol m^{-2} s^{-1}) was considered to be ideal for plant growth in height, width and number of leaves,[63] as applied here for lamb's lettuce growing in the shade (PPFD ~120 μmol m^{-2} s^{-1}).

To investigate the toxicity caused by the plant material grown under higher levels of environmental stress (direct sunlight/PPFD ~400 μmol m^{-2} s^{-1}), MTT viability tests were also conducted with Caco-2 model cells (Fig. 2). Upon 24 h of incubation with the leaf material, Caco-2 cells showed high viability when in the presence of lamb's lettuce cultivars, especially at concentrations lower than 1.0% (w/v) of plant material. Moreover, when assessing cultivars, var. Favor, with a higher content in polyphenols, led to higher percentages of cell viability compared to var. de Hollande. These results also corroborate the well-known chemoprotective effect developed by natural polyphenols on human model cells.[64] Conversely, regarding arugula, higher cell viability levels were observed up to 0.5% (w/v) plant material, especially for *E. vesicaria* var. sativa, which also presented higher amounts of polyphenols/antioxidants. Nonetheless, although lower than for lamb's lettuce, these viabilities agree with previously work focused on the anti-inflammatory,[65] antioxidant, antimicrobial, and anticancer activities of leaf extracts of *E. sativa* on Caco-2 and HCT-116 model cells.[66]

CONCLUSIONS

Global economies need to evolve towards zero emission circular practices. Food production systems based on aquaponics enable the production of plants, seafood and fish by making use of low amounts of nutrient-rich aquaculture water that is fed to plants grown hydroponically, managing the use of water resources, and downsizing the release of ammonium and nitrate into the environment. Ready-to-eat salads and minimally processed vegetables, such as lamb's lettuce and arugula, are among the most consumed foods in the world. A catfish-based aquaponics for producing two cultivars of lamb's lettuce and two cultivars of arugula, at two light intensities (PPFD 120 and 400 μmol m^{-2} s^{-1}), is described in the present study. Because nitrogen contents in fish and plants tend to decrease with increasing fish density, a low fish density of 5.8 kg m^{-3} at a low stocking density of 0.5% (w/w) feed per body weight was applied. The aquaponics system implemented was able to produce all cultivars of lamb's lettuce and arugula, at the same time as maintaining low levels of ammonium (< 0.2 mg NH_4^+–N L^{-1}) and optimal levels of nitrate (~100 mgNO_3^-–N L^{-1}) for plant growth, improving nutrient sustainability and reducing nitrogen-rich wastewater production. At harvest, lamb's lettuce cultivars demonstrated high levels of greenness and health status, particularly the var. de Hollande, which showed higher values of biggest leaf length, root length, foliage diameter, leaf number and plant height, as well as greenness and health status, at both light intensities tested. In turn, arugula cultivars were found to be less light/temperature-tolerant and less adapted to the aquaponics system, as demonstrated by the lower levels of greenness and health status observed, especially the cultivar *E. vesicari*a var. sativa grown under higher light intensity (PPFD ~400 μmol m^{-2} s^{-1}). Hence, the amount of phenolic and antioxidant compounds produced by the plants, as a response to environmental stress, was higher in the specimens submitted to direct sunlight. Nonetheless, the antioxidants/polyphenols produced by the plants were shown to correlate with higher viabilities of human Caco-2 model cells. Thus, the aquaponics system developed in the present study can be upscaled for the future production of lamb's lettuce, as well as for future light/temperature-optimized productions of arugula.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

All methods guaranteed the care and use of animals in compliance with the Legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU & Regulation (EU) 2019/1010).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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