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**Water, energy, and mass transport in sustainable bio-integrated
Recirculating Aquaponics Systems (RAS): Experiments and Computational
Modeling**

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Abstract

This research addressed the study of water, energy, and mass transport phenomena in a sustainable bio - integrated Recirculating Aquaponic System (RAS) coupling the growth of *Oncorhynchus mykiss* (rainbow trout) and *Lactuca sativa* (lettuce). The hypothesis proposed that the energy–environmental sustainability of such sub-systems improves when the transport of fluids, heat, and matter within the production cycle is known, characterized, and modeled; and that integrating engineering, biology, and aquaculture knowledge enhances productivity and product quality while enabling predictive scaling. To test these hypotheses, the study was developed in three stages. First, an exhaustive bibliographic review and experimental characterization of the prototype were performed to quantify the environmental conditions, growth dynamics, and physicochemical variability of each specie throughout the production cycle. Experimental measurements included hydraulic flow rate, dissolved oxygen, pH, temperature, and nitrogen compounds (NH_4^+ , NO_2^- , NO_3^-). These data established the operational baselines and environmental constraints of the system. In the second stage, a physic–biological–mathematical model was formulated to describe the coupled processes of hydrodynamics, heat transfer, and biochemical transformations. The model was derived from the conservation equations of mass, momentum, and energy. The system of partial differential equations described the coupled behavior of water flow, heat transfer, and nutrient dynamics in the aquaponic system. This model was implemented in ANSYS Fluent using CFD and the finite volume method, with a 3D geometry based on the experimental setup. Steady and transient simulations were carried out to predict velocity patterns, temperature distribution, and spatial variations of oxygen and nitrogen species. This research contributed to gain original insights into the quantitative understanding of transport processes in aquaponics, providing a predictive and scalable framework for designing sustainable aquaculture–hydroponic production systems that minimize environmental impacts and maximize resource efficiency.

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CHAPTER 1

ENERGY AND WATER CONSUMPTIONS FOR A SUSTAINABLE AQUAPONIC GROWTH OF TROUT – LETTUCE

1.1 Motivation and relevance

Accelerated global warming has intensified the challenges associated with food security. It has also intensified the challenges associated with access to water and energy sustainability worldwide. Driven by increased greenhouse gas emissions and ecosystem degradation, this phenomenon has created a scenario in which traditional production systems must transition to more efficient, resilient and circular models. FAO and IPCC projections indicate that the global population could surpass nine billion by 2050, leading to a substantial increment in the demand for food, water and energy amid dwindling natural resources.

This issue is particularly acute in arid and semi-arid regions, where water availability is naturally low and extreme weather conditions further reduce agricultural productivity. In regions such as northern Chile, where rainfall is declining, aquifers are being overexploited and there is a competition between the agricultural, urban and industrial sectors, and hence that the urgent need for sustainable water management strategies is clear. In such environments, integrated aquaponic systems offer a promising technological solution for closing water, energy and nutrient cycles. These systems combine aquaculture and hydroponics within a recirculating circuit that minimizes losses and maximizes resource reuse.

Furthermore, both the agricultural and aquaculture sectors are facing mounting restrictions on energy use due to rising energy costs and global decarbonization targets. Studying mass and energy flows within aquaponic systems enables critical efficiency points to be identified and optimization strategies designed to contribute to operational sustainability and a reduced environmental footprint. Understanding the processes of water, energy and nutrient transport is essential not only for improving crop and fish yields, but also for achieving sustainable and scalable productive integration.

This research is driven by the necessity to produce applicable and quantifiable knowledge regarding the internal dynamics of bio-integrated aquaponic systems through controlled experimentation and computational modelling. This approach aims to strengthen the scientific basis for the development of sustainable recirculation technologies, particularly adapted to the conditions of water and energy scarcity that characterize the arid and semi-arid regions. The primary objective of this study is to contribute to the design of key food production systems that integrate efficiency, resilience and environmental sustainability. This will help to address the global challenges of climate change and the increasing pressure on natural resources.

1.2 Proposed research

This research proposes to evaluate the sustainable growth of rainbow trout (*Oncorhynchus mykiss*) and lettuce (*Lactuca sativa*) in a bio - integrated recirculating aquaponic system by a Life Cycle Assessment (LCA) that allows to estimate the energy and water consumptions and the environmental footprint.

1.3 Hypothesis and Goals

The two hypotheses of this thesis are:

- Energy-environmental sustainability in the growth of trout and lettuce, in a recirculating aquaponic system, improves if the transport phenomena of fluids, heat and matter in the productive cycle are known and characterized.
- Knowledge integration of engineering, biology and aquaculture in water, energy and nitrogen compounds flow prediction improves productivity and quality of the recirculating trout-lettuce aquaponic system, generating original results for productive scaling up in a sustainable way for food in semi-arid and arid regions.

The General goal is:

- To develop and validate a methodology based on computational modelling to determine the flows of energy, water and matter in a sustainable aquaponic system for the cultivation of trout and lettuce, based on the fluid mechanics and energy governing equations solved by the finite volume method (FVM). The main objective is to relate fluid, heat and mass transport phenomena to the growth of trout, and vegetables in an experimental aquaponics system that uses energy and water resources efficiently and sustainably in a semi-arid climate.

Specific goals:

- Evaluate the performance of a prototype of a recirculating aquaponic system for the growth of trout and lettuce, to understand the physical and biological phenomena that occur in the fish-bacteria-plant integrated system
- Build a physical-biological-mathematical model for an aquaponic system for trout and lettuce growth located in a region with a semi-arid climate.
- Predicting the time variation of the distribution of velocity, temperature, and concentration of nitrogen compounds in fish and lettuce culture ponds using computational simulations with the MVF
- Analyze and discuss the results of fluid mechanics, heat and mass transfer in the integrated recirculating aquaponic system during the production cycle.
- Evaluate the energy efficiency and environmental sustainability of the integrated computational model for recirculating fish-bacteria-plant aquaponic culture.

1.4 Methodology

This research was conducted using a mixed approach, combining experimental, and computational methods, with the aim of understanding and predicting the phenomena of water, energy, and mass transport in recirculating aquaponic systems that integrate the production of trout (*Oncorhynchus mykiss*) and lettuce (*Lactuca sativa*). The interdisciplinary nature of the study involved the integration of knowledge from engineering, biology and aquaculture, with an emphasis on physical modelling and numerical simulation of coupled the processes.

The methodological design was structured in three main stages: (1) literature review and experimental characterization of the system, (2) development of the physical-biological-mathematical model, and (3) computational simulation and experimental validation. These stages were carried out sequentially and complementarily, allowing progress from empirical observation and theoretical analysis to the formulation and validation of predictive behavior models.

The quantitative approach was achieved by the measurement and statistical analysis of physical-chemical, thermal, and biological parameters, while the qualitative dimension was present in the interpretation of process dynamics and the interdisciplinary integration of knowledge. The applied nature of the study was evident in the orientation of the results towards the food production optimization and the environmental sustainability of bio - integrated systems.

1.5 Thesis structure

This doctoral thesis is composed of five chapters, each addressing a specific dimension of water, energy, and mass transport processes in sustainable bio-integrated aquaponic systems.

The first chapter, titled “*Energy and Water Consumption for a Sustainable Aquaponic Growth of Trout–Lettuce,*” introduces the motivation and scientific relevance of the research. It presents the main hypotheses, objectives, and methodological framework that guided the study, emphasizing the need to understand the coupling

between hydrodynamics, energy use, and biological performance in recirculating aquaponic systems.

The second chapter, “*Water Use in Lettuce Production in Semi-Arid Regions*,” explores the challenges of efficient water use in agriculture under semi-arid conditions, where water scarcity poses a serious threat to food security and sustainable production. This section contextualizes aquaponics as an alternative technology capable of reducing water consumption through nutrient recycling and controlled recirculation.

The third chapter, “*Lettuce Growth in Hydroponic Beds Using the Deep-Water Culture (DWC) Technique*,” examines the effect of implementing an innovative pulsatile water flow strategy designed to mitigate the spatial variability within the cultivation beds. Through experimental trials, this chapter demonstrates how hydraulic modulation can improve the crop uniformity in size, nutrient availability, and water quality management within DWC systems.

The fourth chapter, titled “*Trout and Lettuce Growing Performance in a Closed-Loop Aquaponic System*,” evaluates the biological responses of both species under the integrated conditions found at the experimental facility. It analyzes how nutrient dynamics, water quality, and recirculation parameters influence the growth rate, feed conversion ratio, and biomass yield of trout, as well as the physiological performance and nutrient uptake efficiency of lettuce.

Finally, the fifth chapter, “*Sustainability of the Trout–Lettuce Aquaponic System through Life Cycle Assessment (LCA)*,” assesses the overall environmental performance of the integrated system under controlled greenhouse conditions in northern Chile’s semi-arid region. Using a gate-to-gate Life Cycle Assessment, the study quantifies the environmental indicators such as Water Use Efficiency (WUE), Specific Energy Consumption (SEC), Cumulative Energy Demand (CED), and Global Warming Potential (GWP) across a nine-month production cycle.

CHAPTER 2

LETTUCE GROWTH IN HYDROPONIC BED USING DEEP WATER CULTURE TECHNIQUE (DWC)

2.1 Abstract

Hydroponic systems represent a sustainable and efficient alternative for crop production in the present scenery of global challenges such as climate change, population growth, and the progressive reduction of water and energy resources. Despite their advantages, these systems often exhibit spatial variability in plant growth, uneven nutrient distribution, and temperature accumulation, which together reduce their productive efficiency and stability. These issues become particularly evident in systems that operate with continuous water flow, where the lack of hydraulic balance leads to localized variations in water quality and nutrient availability.

The present study proposes the evaluation of innovative pulsatile water flow strategies as a means to mitigate spatial variability of nutrients and improve both crop uniformity and water quality management in Deep Water Culture (DWC) hydroponic systems. Experiments were conducted under greenhouse conditions during summer and autumn, comparing three hydraulic configurations: a Continuous Water Flow (CWF) system with 24-hour operation and a single inlet, a Pulsatile Water Flow (PWF) system with intermittent operation through a single inlet, and a Three-Inlet Pulsatile Water Flow (TWF) system distributing the flow in three points to enhance circulation. The analysis included parameters such as lettuce growth distribution, water temperature, nutrient retention time, and the occurrence of border effects.

The results showed that the CWF configuration produced higher water temperatures, reduced nutrient retention, and marked differences between central and peripheral plants, confirming a strong spatial imbalance. In contrast, both pulsatile systems (PWF and TWF) achieved lower water temperatures, longer nutrient retention times, and more uniform nutrient availability, leading to homogeneous lettuce growth

throughout the cultivation area. The TWF system demonstrated the best overall performance, providing consistent nutrient distribution and improved crop uniformity. Compared to conventional methods, the pulsatile configurations allowed for an 80% reduction in water use and a 25% decrease in the production cycle duration, originating significant advances in efficiency and sustainability. These findings demonstrate the potential of pulsatile water flow to optimize hydroponic management and reduce the environmental impact. Although the first applications were carried out by small-scale farmers in northern Chile in collaboration with the Agricultural Research Institute (INIA), the scalability and adaptability of this approach position is a promising technological innovation for a sustainable agriculture in arid and semi-arid regions around the world.

2.2 Literature review

As an alternative to traditional agriculture, soilless cultivation techniques are currently fast being developed, including hydroponics, which is a method of growing plants in a nutrient-rich water solution without soil (Kozai *et al.*, 2022). The crop yields of cultivation of lettuce, chicory, and spinach have been quantified by a global meta-analysis (Goh *et al.*, 2023), where controlled environment agriculture (CEA) was observed to produce significantly higher crop yields compared to Open-Air Agriculture (OAA) for certain crops, emphasizing the importance of growing environments in hydroponic farming.

Hydroponic variants include the Nutrient Film Technique (NFT), where a thin film of nutrient-rich water continuously flows over the roots of the plants, providing them with the essential nutrients and oxygen (Lennard and Leonard, 2004). Another method is Deep Water Culture (DWC), in which the plant roots are suspended in a nutrient-rich solution, with the roots submerged in water. In this technique the oxygen is provided using air stones or diffusers (Nursyahid *et al.*, 2021).

Solar radiation and air temperature have also been recognized as important parameters for lettuce growth in hydroponics, which in terms of lettuce biology are

dependent on the seasonal variations. In a study developed by (Glenn, 1984), the interaction of day temperature and radiation were correlated with leaf growth in lettuce and harvest time. In those experiments, highest temperatures, during the summer rainy season, were associated with bolting, which negatively affected the leaf lettuce growth. In addition, plant harvesting times varied significantly, ranging from 42 days in summer, with head weights of 159 grams, to 62 days in winter, with head weights of 20 grams. In general, weather seasons significantly impact lettuce cultivation, especially in terms of temperature. High tunnels can help to mitigate the adverse weather conditions, providing a controlled environment for lettuce growth (Jayalath *et al.*, 2017).

Lettuce growth is sensitive to extreme temperatures, with cold temperatures between -10 °C and -17°C negatively affecting lettuce cultivation in high tunnels (Mihai *et al.*, 2014). At least two parameters: light intensity and air temperature are key factors influencing lettuce growth and development, with optimal light values varying at different temperatures for lettuce production (Tarr *et al.*, 2023; Wheeler *et al.*, 1993; Zhou *et al.*, 2019). These diurnal and nocturnal temperature variations also influenced the nutritional composition of the lettuce significantly (Gent, 2016).

Hydraulic conditions govern hydroponic cultivation, where regulating the flow rate is crucial in achieving the desired hydroponic crop quality (Intriago *et al.*, 2018). Baiyin *et al.*, (2021b) reported that the optimal flow rate for hydroponic plant growth of Swiss chard plants was between 2 and 6 L/min. This range promoted root growth, nutrient absorption, and overall plant growth. Thus, suitable water flow rates within this range act as a positive stress (eustress), providing appropriate mechanical stimulation to the roots and facilitating higher nutrient absorption (Baiyin *et al.*, 2023; Baiyin *et al.*, 2021; Dalastrá *et al.*, 2020). Instead, excessive flow rates, particularly in the range of 6-8 L/min, act as distress for the plants, inhibiting root surface area and overall root growth, where compacted roots provokes a reduced nutrient absorption (Baiyin *et al.*, 2021b).

Amin *et al.* (2022) compared the nutrient consumption rates and plant growth of different hydroponic systems with varying flow rates. The gutter hydroponic system

with a flow rate of 1.5 L/h exhibited the highest nutrient consumption rate and whole plant weight. It also had the highest values of N, P, K, Ca, and Mg consumption rate. The water use of lettuce plants in this system was 46.11 kg/m³. Also, the DWC hydroponic system with a flow rate of 2.0 L/h had the lowest nutrient consumption rate, while the highest nitrogen consumption rate was found with a flow rate of 1.5 L/h. The water efficiency of lettuce plants in this system varied depending on the flow rate, with the lowest efficiency at 1.0 L/h and the highest one found at 1.5 L/h.

Uneven growth in hydroponic plants is heavily influenced by factors such as nutrient distribution, salinity levels, and environmental conditions, all of which play a key role in determining overall yield and plant health. Variations in nutrient concentration and salinity often result in differential growth responses, creating disparities among plants within the same system (Hofmann et al., 2024). Nutrient distribution, for instance, directly affects physiological processes and can lead to disorders such as blossom end rot (BER) in tomatoes. Research findings have shown that high electrical conductivity (EC) solutions increase the incidence of BER, while lowering EC reduces it by up to 80%, as observed by (Tabatabaie et al., 2004). Additionally, uneven EC treatments alter the concentration of essential nutrients in fruits, leading to higher calcium (Ca) and lower potassium (K) levels, further influencing the plant development.

Salinity stress is another critical factor, with studies on "Biquinho" pepper plants demonstrating that growth variables decrease linearly as the salinity levels rise (Cova et al., 2022). However, moderate salinity levels have been found to induce acclimatization, eventually improving plant growth over time. Proper management of salinity, including the strategic addition of calcium and potassium, has been shown to mitigate growth reductions, underscoring the importance of nutrient additives in maintaining plant health under saline conditions (M. Koushifar and A. Khoshgoftarmanesh, 2015). Beyond nutrient and salinity management, maintaining a uniform environment is essential for consistent growth in hydroponic systems. Experiments in controlled environment rooms (CER) have demonstrated that

suitable temperature levels and nutrient concentrations significantly minimize growth variability among plants (York et al., 1987).

One of the novelties of this study lies in report the finding of a pronounced edge effect on plant growth when implementing classical water circulation techniques (CWF) compared to homogeneously significant growth across the length and width of the hydroponic bed when implementing pulse flow strategies (PWF and TWF).

A unique pulsatile water circulation technique (PWF) is investigated, where water flows in controlled pulses rather than continuously, alongside a three-point inlet system (TWF) to optimize nutrient distribution and water quality in Deep Water Culture (DWC) hydroponics. Designed to perform well under high-temperature conditions, this approach demonstrated remarkable improvements over conventional continuous water flow (CWF) systems. Unlike CWF, which raises water temperatures through constant 24-hour movement, PWF minimizes temperature increment by reducing the kinetic water activity to only 14% of CWF's, extending the nutrient solution residence time to improve the root absorption. Notably, the TWF system enhanced these effects, achieving a shorter crop cycle than both CWF and PWF, with growth improvements of up to 220% during winter in the southern hemisphere.

The role of water flow strategies in the spatial variability of lettuce growth across hydroponic beds is critical, as it directly influences the uniformity of crop development. Notably, the innovative Pulsatile Water Flow (PWF) and Three-Inlet Pulsatile Water Flow (TWF) systems significantly reduced the border effect at the bed lateral boundaries, a phenomenon where growth near the water inlet differs from other zones in the bed, compared to the Continuous Water Flow (CWF) system. By maintaining a uniform water quality and nutrient distribution throughout the bed, PWF and TWF minimized spatial disparities in plant growth, with TWF achieving the highest levels of uniformity. These findings highlight the scalability and efficiency of pulsatile flow strategies, particularly TWF, which not only optimize the plant growth but also improve resource use efficiency across the entire growing area, presenting a promising approach for sustainable hydroponic cultivation.

Currently at the Agricultural Research Institute (INIA, Chile) these improvements are implemented with small farmers, that are part of the most vulnerable group in the agricultural sector (peasant family agriculture, Rural Family Agriculture (RFA). Preliminary results indicated that the main achievements found were a reduction of 70% in the use of land surface; a 400% increment in productivity; reductions of 80% in water usage and 25% in the production cycles compared to soil agriculture. These improvements were implemented through technological transfer instances, that generated direct economic benefits for producers.

2.3 Goal of the study

The main objective of this research is to evaluate, from both an experimental and analytical perspective, the effectiveness of innovative pulsatile water flow strategies in Deep Water Culture (DWC) hydroponic systems, aiming to reduce spatial variability in plant growth and to enhance the transport dynamics of water, energy, and nutrients within the production environment. The study seeks to understand how controlled hydraulic pulsations, implemented through single and multiple inlet configurations, can improve the homogeneity of physical and chemical parameters in the water column, such as temperature, dissolved oxygen, pH, and nutrient concentrations, which directly influence crop performance and system efficiency.

2.4 Methodolgy

2.4.1 Experimental design

Lettuce of the Quenty variety was grown using the deep-water culture hydroponic technique (DWC), with water recirculation. Quenty is a type of Oakleafe lettuce, having compact, uniform, and a bright light green color, with strong post-harvest characteristics resistant to *Bremia* and tolerant to viruses (Crute and Norwood, 1981), being highly recommended for hydroponics. DWC technique is based on the development of vegetable crops in water with a nutritive solution, on polystyrene, excluding the use of any type of substrate (Nursyahid et al., 2021).

The experiments were conducted in a 177 m³ experimental greenhouse facility at *Instituto de Investigaciones Agropecuarias, INIA Intihuasi*, La Serena, Chile. This greenhouse was constructed using a three-layers polyethylene, 130 microns thick, with two-season UV additive and 89% light transparency. The coordinates of the facility are -29° 91'90" S, -71° 24'84" W. Relevant growing parameters, including air temperature, solar radiation, and relative humidity, were measured every 15 minutes using a Sensor ATMOS 14 and stored in a datalogger.

The lettuce seedlings were contained in three DWC hydroponics beds, with a capacity of 300 liters of water each one (300 x 100 x 10 cm), built in wood and lined on the inside with a 4 mm thickness plastic. Figure 1 illustrates the scheme of the three hydroponics beds, made of six high density polystyrene trays, covered in black plastic, with a capacity of 15 seedlings in each tray, and a total of 90 seedlings contained in each bed.

One of the hydroponics beds operates 24 hours per day with a continuous recirculating flow water mode (CWF), and one water inlet. A second one operated every day, from 8:00 to 18:00 hours, with a pulsed flow water recirculation mode, six times a day for 20 minutes per pulse, entering through a single inlet (PWF), operated by 120 effective minutes. The third bed consisted of a pulsating water recirculation system, with the same frequency used in PWF, which was functioning simultaneously to the CWF and PWF. In TWF the water inlet was through three zones of the bed, as depicted in Figure 1. The black squares illustrate the position where water quality was measured.

The inlet velocity, flow duration and time interval of each water flow mode is detailed here. A total of six pulses, with duration of 20 minutes each one, were imposed for the PWF and TWF modes. Fluid recirculation was accomplished daily by pumping water in the time interval between 8:00 and 18:00 hours. The three water circulation modes of 0.17 L/min, with an inlet velocity of 0.004 m/s in all cases, were performed through a plastic hose with an inner diameter of 1/2", during all the lettuce growth period.

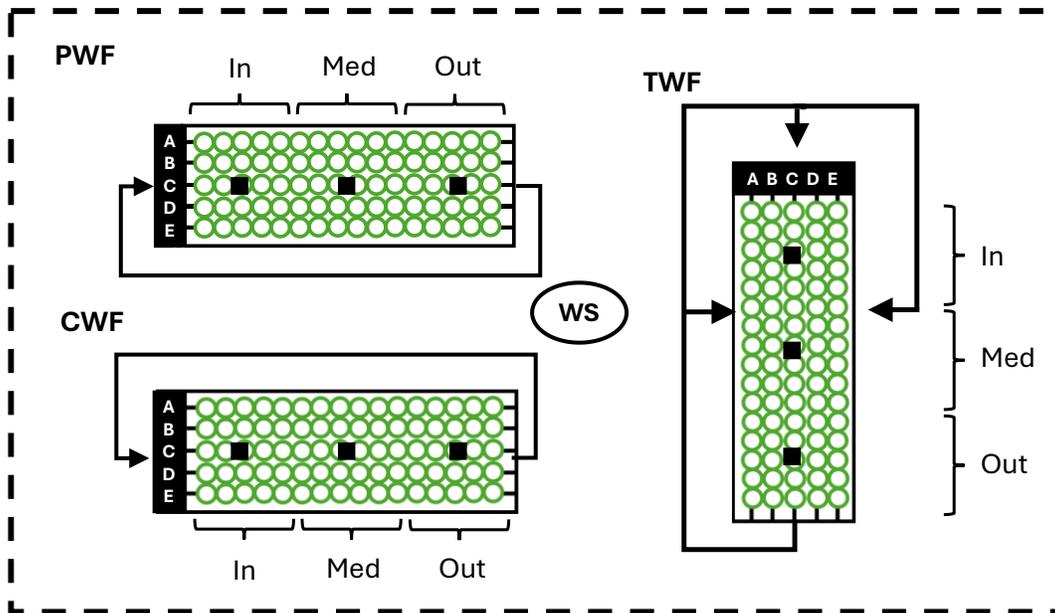


Figure 1. Inlet water arrays used in the lettuce growing experiments along the hydroponic beds for: (a) CWF at continuous flow, (b) PWF at pulsatile flow and (c) TWF operated as a pulsatile flow with three water inlets. “WS” indicates the zone where the weather station was located. The pulsed modes operated for 2 hours during the daytime, while the continuous mode operated each day for 24 hours.

These situations and flow variation were studied for two climatic seasons in the southern hemisphere: first, was evaluated in summer from March 1 to April 3, 2023. The minimum external air temperature recorded during this period was 12.8°C, while the maximum temperature reached 22.6°C. The average external air temperature during the 33 days period was 17.2°C. The air temperature inside the greenhouse in the growing stage was 25.9 ± 3.2°C. Solar radiation inside the greenhouse varied between 0 and 340 W/m².

A second experiment that spanned 26 days was accomplished in autumn, from April 22nd to May 23rd, 2023. The external air temperature during this period ranged from a minimum of 8.72°C to a maximum of 22.2°C, with an average value of 13.9°C, while the solar radiation inside the greenhouse varying from 0 up to 178 W/m².

Each hydroponic bed was treated with the same nutrient solution, based on the formulation of a modified solution (Steiner, 1961), that was added only once, at the

beginning of the transplant, and remained recirculating in each bed during the entire course of the experience.

The nutrient solution was prepared using concentrated fertilizers in two separate phases. The quantity of the nutrients was diluted in 10 liters of water, from which 1.5 liters were applied for each 300-liter hydroponic bed. In the first phase, 100 grams of potassium sulfate, 43 ml of 80% concentrated phosphoric acid, 50 grams of magnesium sulfate, and 17 grams of iron chelate were used. The second phase, applied and diluted after the first, includes 450 grams of calcium nitrate and 500 grams of potassium nitrate.

2.4.2 Environmental quality measurement

To characterize the variation of environmental conditions in these two periods, a remote weather station was used to measure air temperature at 1.5 [m] altitude, relative air humidity and solar radiation, at the same altitude, inside the greenhouse, using sensors QSO-S Par and Atmos 14 to ambient temperature ($\pm 0.1^\circ\text{C}$), relative humidity ($\pm 0.1\%$) and solar radiation (± 1 Lux). DWC were kept isolated inside the greenhouse, maintaining automated control and registry, every 15 minutes, with a meteorological station, measuring the internal temperature, air humidity and solar radiation.

2.4.3 Growing Degree Days (GDD)

The GDD of the air inside the greenhouse was calculated using the maximum and minimum air temperature being recorded daily at the weather station. The equation used corresponds to the one modified by McMaster and Wilhelm (1997).

$$GDD = \frac{T_{max} + T_{min}}{2} - \log_{10}(T_{base}) \quad (1)$$

where GDD is the degree-day accumulation in base 10, T_{max} is the maximum daily temperature in degrees Celsius, T_{min} is the daily minimum temperature in degrees Celsius, and T_{base} is the base temperature in degrees Celsius, defined as the threshold value below which the number of degree-days accumulated.

2.4.4 Water quality measurement

Water quality variables such as temperature (± 0.5 °C), pH (± 0.05), and electrical conductivity (± 0.01 mS/cm) in the water were monitored manually daily at midday, with a Hanna HI98130 equipment. Measurements were made at three zones of the hydroponic bed, at 5 cm water depth with respect to the bed bottom. Water samples were taken in these areas to measure the dissolved oxygen in the water (± 0.01 ppm/L O_2), which were processed in a Hanna HI2400 meson equipment.

2.4.5 Nutrients in water

During both crop cycles, in summer and autumn, the concentration of nutrients in the water was measured by taking water samples twice a week, at the central zone (zone 2), and at mid-water level of each hydroponic bed.

The samples were processed in the laboratory, using a visible light spectrophotometer, model IRIS HI801. Nutrients analyzed were Calcium (mg/L Ca^{2+}), Nitrate (mg/L NO_3^- -N), and Alkalinity (mg/L $CaCO_3$). The accuracy and method used in the spectrophotometer for the nutrients is detailed in Table 1.

Table 1. Nutrients measured by visible light spectrophotometry.

Parameter	Range	Accuracy (at 25°C)	Method
Alkalinity	0–500 mg/L $CaCO_3$	± 5 mg/L $\pm 5\%$ of reading	Bromocresol green
Calcium	0–400 mg/L Ca^{2+}	± 10 mg/L $\pm 5\%$ of reading	Oxalate

Nitrate	0.0–30.0 mg/L N–NO	± 0.5 mg/L $\pm 10\%$ of reading	Cadmium reduction
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2.4.6 Root and leaf length measurement

The root length was measured using a digital vernier, with an accuracy of ± 0.01 [mm]. This procedure was applied twice a week to all the plants of the CWF, PWF and TWF bed systems. The length of the leaf was measured from the central leaf to the plant neck, in order not to stress or manipulate the plants, as illustrated in Figure 2, without removing the plants from their substrate.

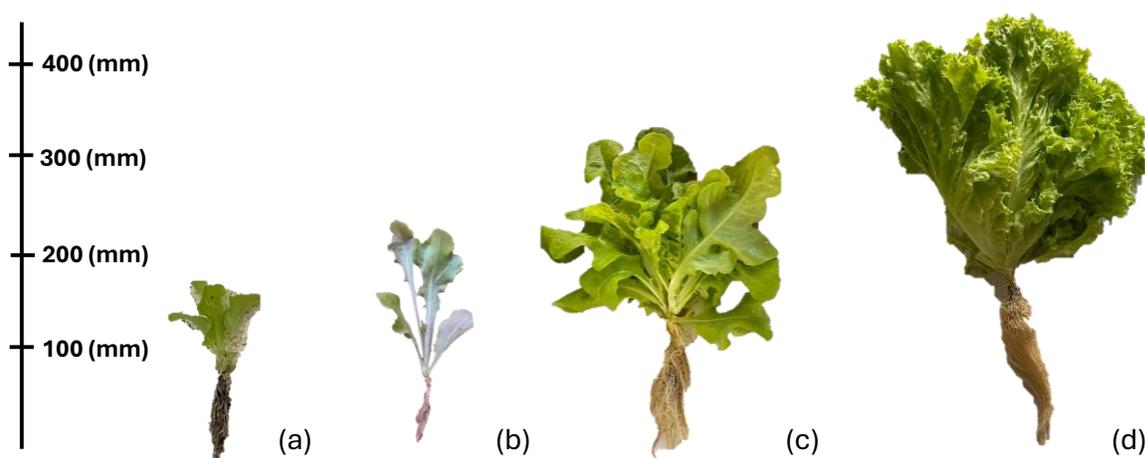


Figure 2. Growth indicator, leaf length, measured in cultivated lettuce, showing the weekly evolution of growth in mm, at: (a) week 1, (b) week 2, (c) week 3 and (d) week 4.

2.4.7 Fractional Green Canopy Cover (FGCC) measurement

The images for this technique were obtained from aerial photography, supported on a monopod type structure, arranged at a height of 1 meter with respect to the hydroponic bed. The photographs were taken with a smartphone Iphone model 11S, by manual adjustment of its capture settings, with a wide-angle camera of 26 mm f1.8, and a shutter speed of 1/213 seconds. The photographs were analyzed with the Canopeo® software. The FGCC derived from Canopeo was useful for estimating the biomass yield, by aerial photographs, which represents the proportion of the

ground covered by green foliage (Reed *et al.*, 2021). Canopeo® is a software tool that serves the purpose of quantifying the FGCC in images. Developed using MatLab, this application employs the RGB color system and operates on the principles of colorimetry (Patrignani and Ochsner, 2015). This tool has been used for the determination of FGCC in leafy green crops as well as in other vegetables. The accuracy of this technique has been found to be reliable under varying light and environmental conditions, by a strong association with NDVI, indicating the correlation of a visual index (FGCC) and a spectral index (Tenreiro *et al.*, 2021). The photographs were taken at two sections of each hydroponic bed; in which FGCC in corresponds to the first three plates and *FGCC out* refers to the last three plates, allowing to determine whether the FGCC showed differences depending on the location in the bed.

2.5 Results and discussion

2.5.1 Environmental data

The daily average results obtained from the analysis of the environmental conditions inside the greenhouse, in the seasons of study, are presented in Figures 3 to 6, observing a strong and inverse relationship between the air temperature inside the greenhouse and the environmental humidity in both climatic seasons. This relationship and the factors that determine these heat transfer phenomena and their effects on the crops were analyzed by Suzuki *et al.*, (2015) who observed in tomato greenhouses that the temperature inside the greenhouse influences humidity levels, with higher temperatures that can lead to increased evaporation and higher humidity, while lower temperatures result in lower humidity levels.

In this study, in summer, in summer, the minimum external air temperature was 12.8°C, the maximum one was 22.6 °C, and the average temperature was 17.2°C, while the solar radiation varied between 0 and 340 W/m². The Spearman correlation coefficient was -0.9681, with a 95% confidence interval from -0.9844 to -0.9352, indicating a very strong negative correlation between the variables. (Fig. 3).

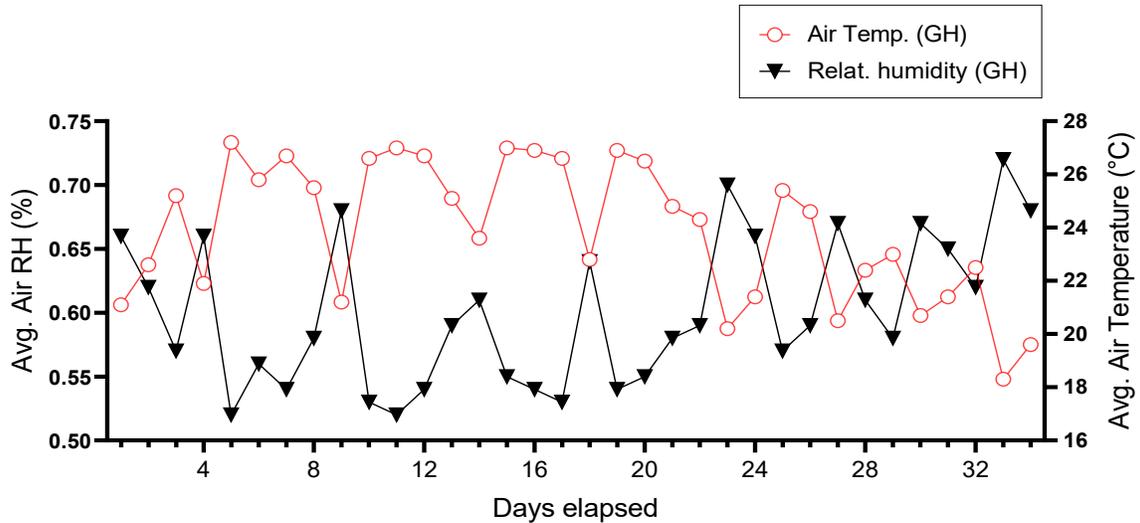


Figure 3. Evolution of relative humidity and average air temperature inside the greenhouse in summer during lettuce growth.

In contrast, in autumn the average air temperature inside the greenhouse had a minimum value of 8.72 °C, with a maximum value of 22.2 °C, and a variation of solar radiation in autumn that was between 0 up to 178 W/m² (Fig. 4). The Spearman correlation coefficient between the variables was -0.5960, with a 95% confidence interval ranging from -0.7772 to -0.3236, indicating a moderate negative correlation in autumn. The solar radiation has a significant effect on the relative humidity inside greenhouses. In the presence of solar radiation, the air temperature and relative humidity in the greenhouse may vary drastically (Deng *et al.*, 2023). On the other hand, natural ventilation reduces the humidity ratio of indoor air in the solar greenhouse (Fan *et al.*, 2023). In our study, ventilation inside the greenhouse was not employed.

2.5.2 Growing Degree Days (GDD)

In summer the evolution of the vegetation cover fraction (FGCC) was evaluated as a function of the accumulated degree days (GDD) of the air inside the greenhouse, as shown in Figure 5.

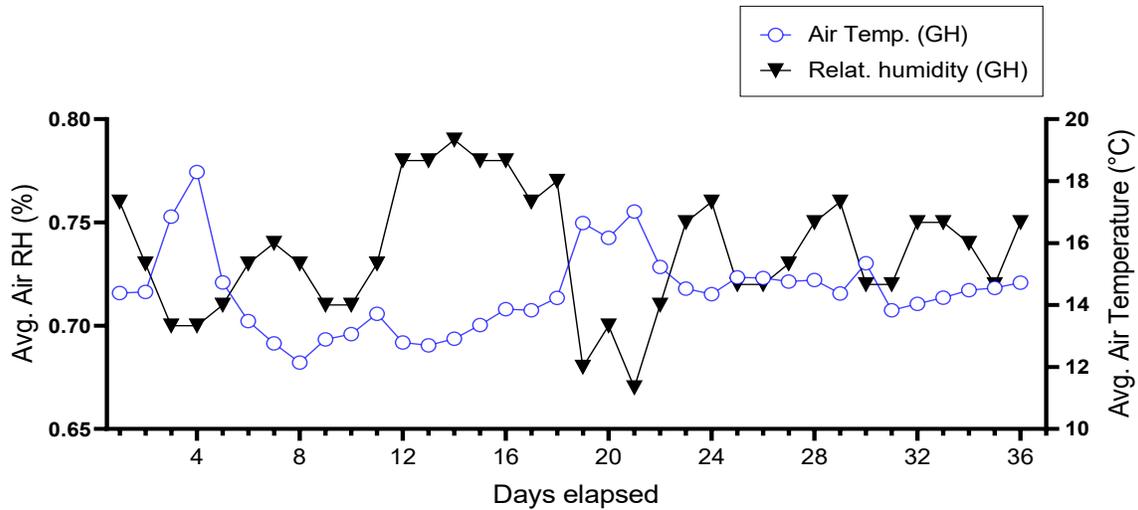


Figure 4. History of relative humidity and average air temperature inside the greenhouse in autumn.

The analysis of the results obtained allows to infer two stages of growth of this crop associated with GDD, an initial stage characterized by lower growth rates for both systems (lower slope of the curves) and a final phase with accelerated growth, demonstrated by a greater earring. The PWF system tends to show a higher FGCC compared to the CWF, suggesting that the PWF allows better crop development.

A highlight on these results occurs when a maximum temperature of 28°C was recorded after 18 days. This event seems to be critical, since the PWF system shows a clear advantage in terms of FGCC over the CWF. This difference could be related to a better availability and absorption of nutrients in the PWF system, which favors the crop growth.

As in summer, in autumn an initial stage of slow growth was observed in the three cultivation systems. After 78 GDD, differentiation in growth (FGCC) is observed in Fig. 6, being higher for TWF. After 20 days of cultivation, the maximum temperature of 25.7 °C was observed inside the greenhouse, which affected the CWF and PWF systems, causing a decrease in lettuce growth, a situation that affected the crop until harvest.

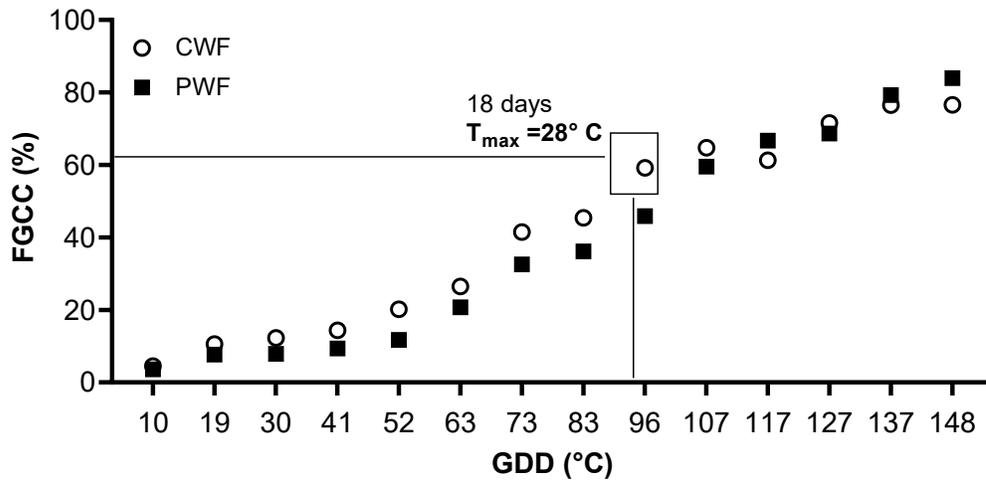


Figure 5. Relationship between the increment of lettuce fraction green cover (FGCC) and the growing Degree Days (GDD) inside the greenhouse in summer.

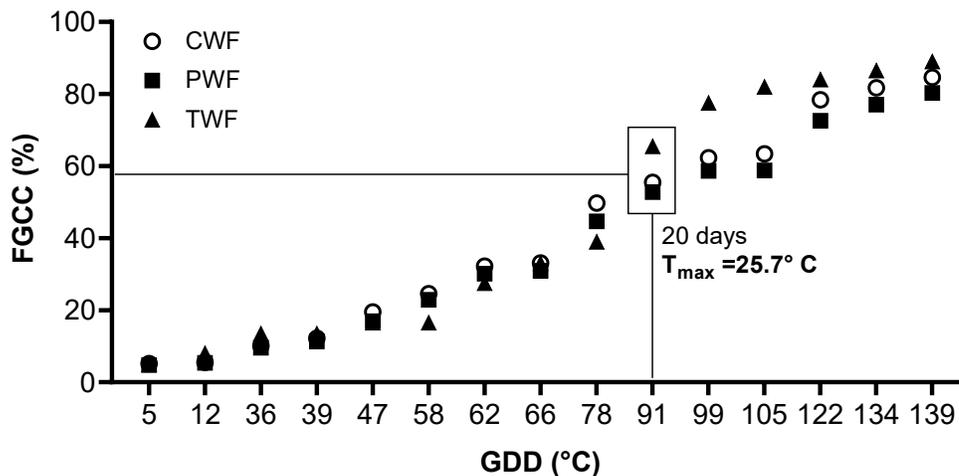


Figure 6. Relationship between the increment of the fraction green cover (FGCC) and the growing Degree Days (GDD) of lettuce inside the greenhouse in autumn.

2.5.3 Average Water Temperature and pH

Water temperature, oxygen, pH, and FGCC were analyzed by averaging the measurements at the zones indicated in Figure 7, in each hydroponic bed, for both summer and autumn. The results of water temperature in the beds indicated that the

effect of the environmental fluctuations of the greenhouse on the water was dampened, finding for summer an average difference between the outside ambient temperature and inside the greenhouse of 7.5 ± 2.9 °C. The average air and water temperature inside the beds registered a difference of 4.1 ± 3.8 °C. Ahn *et al.* (2015), found that the temperature inside greenhouses can be significantly higher than the temperature outside.

In autumn, the difference in temperature of air inside the greenhouse and outside was 3.5 ± 1.2 °C, while the difference between air temperature inside the greenhouse and the average water temperature was 5.2 ± 1.6 °C. The difference between the outside and inside temperature of a greenhouse can vary depending on various factors such as ventilation, side vent heights, and the specific design of the greenhouse, where higher side vents resulted in a decrease in temperature differences between inside and outside the greenhouse, (Seo *et al.*, 2021). In our study, the greenhouse remained constantly closed and no ventilation strategies were employed. In contrast, Khaoua *et al.*, (2006) highlighted the impact of various opening configurations on natural ventilation, internal microclimates, air circulation, and climate heterogeneity within a greenhouse. They emphasized the importance of considering parameters such as air velocity, temperature profiles at plant level, and climate heterogeneity to determine the most effective ventilation configuration for optimal greenhouse conditions.

Natural ventilation plays a vital role in creating a sustainable and healthy indoor environment for humans and plants alike (Roberts, 2003), by facilitating the exchange of carbon dioxide, regulating temperature, and reducing humidity levels. Natural ventilation contributes significantly to maintaining a comfortable atmosphere for plant growth (Chen *et al.*, 2021; Jin *et al.*, 2013). This natural process has generated an increased attention from scholars, particularly in the realm of greenhouse technology, highlighting its importance in achieving high standards in environmental regulation and energy conservation. The emphasis on natural ventilation as a cornerstone of greenhouse technology underscores its indispensable value in fostering sustainable indoor environments (Seo *et al.*, 2021).

Finally, the water temperature in the CWF flow condition was 1.2 ± 0.4 °C higher than in PWF. The most relevant finding in this regard was the temperature difference between water in PWF and CWF condition, where throughout the whole experience in summer the CWF system showed an averaged value that was 1.3 ± 0.4 °C higher than in the PWF system (Fig. 7). This could be explained by the higher fluid dynamics of water in the CWF condition.

As for the evolution of pH, when analyzing its dynamics, a strong negative correlation was observed between pH and CWF, with a value of -0.91 for CWF and -0.95 for PWF. This is related to the factor that CWF is an indirect way to evaluate the growth of lettuce in hydroponics, also impacting on several growth parameters, such as number of leaves, fresh weight, and the number of leaves (Frasetya et al., 2021).

In both systems, during summer and autumn, pH showed a strong fall when the nutrient solution was added, dropping by 2 units. During the summer experiments, the drop in pH was slow, but sustained over time, reaching pH levels of 3 for CWF and PWF at the end of the cycle. This drop was more pronounced in autumn. During the first two weeks of the cycle the value of pH remained constant.

In addition, when analyzing the evolution of the pH in the water inside the beds in summer, for CWF and PWF, and comparing the results with the evolution of the FGCC parameter, an inverse relationship was found. In addition, the pH in the water, for these growing conditions, followed a similar pattern that the oxygen in the water (Fig. 8). This relationship, and its impact in hydroponics systems has not been studied, however, in aquaculture has been reported that there is a significant positive correlation between pH and dissolved oxygen, especially in waters with a high exchange rate during summer and autumn (Zang et al., 2011).

The pH of water in the northern region of Chile ranges between 7.5 and 8.5. When using the nutrient solution, the pH dropped to levels between 5.0 and 5.5, which are the acidity levels required to the absorption of most essential nutrients by plants and prevent the precipitation of Ca and Mg with HPO_4 and the immobilization of P, Mg, and Fe. Therefore, the common practice in hydroponics is to maintain the pH at

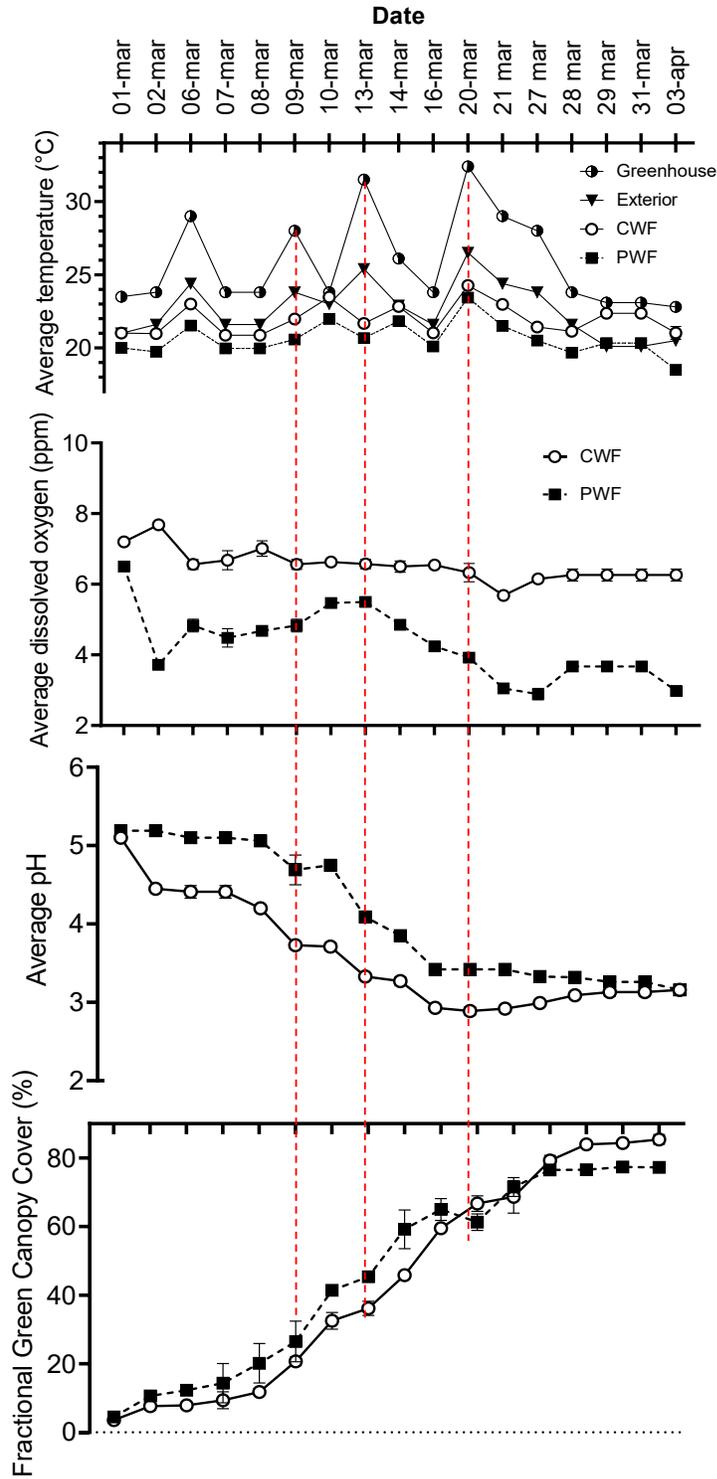


Figure 7. Evolution of average air and water temperature, for the CWF and PWF inlet flow conditions, and their relationship to pH and dissolved oxygen in Summer.

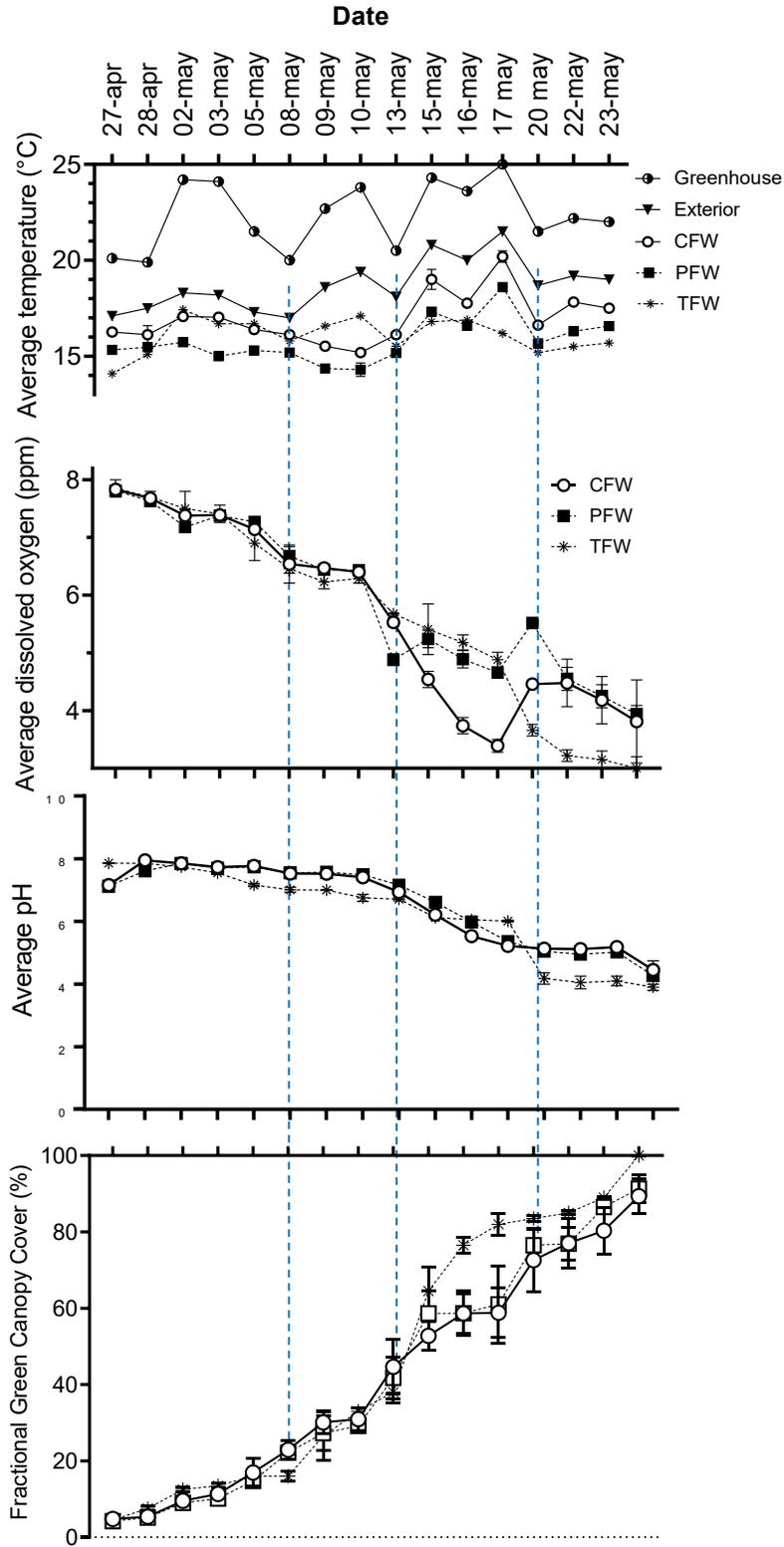


Figure 8. History of average air and water temperatures, for CFW, PFW and TFW inlet flow conditions, and their relationship to pH and dissolved oxygen in Autumn.

these levels. In this study, the pH was not modified; hence, the trend reported in Fig. 9 is a result of ambient CO₂ levels and the rate of nutrient absorption, that is in agreement with previous results reported by Concepcion *et al.* (2022). The modulatory effect of pH in autumn followed the same trend as in summer, where a strong negative correlation was observed between pH and FGCC; with a value of -0.95 for CWF, -0.94 for PWF and -0.93 for the TWF water inflow configuration.

The evolution of the parameters for CWF, PWF and for TWF in summer and autumn, have a relevant spatial component when analyzing lettuce growth results. Distribution of average water temperature and pH is analyzed by zones ('in', 'med' and 'out'). In summer, dissolved oxygen distribution over time was influenced by the ambient temperature variations as described in Figure 7. The weekly averages for CWF (Fig. 9a) were higher than in PWF (Fig. 9b), and the distribution in the near inlet, middle and outlet zones did not differ significantly.

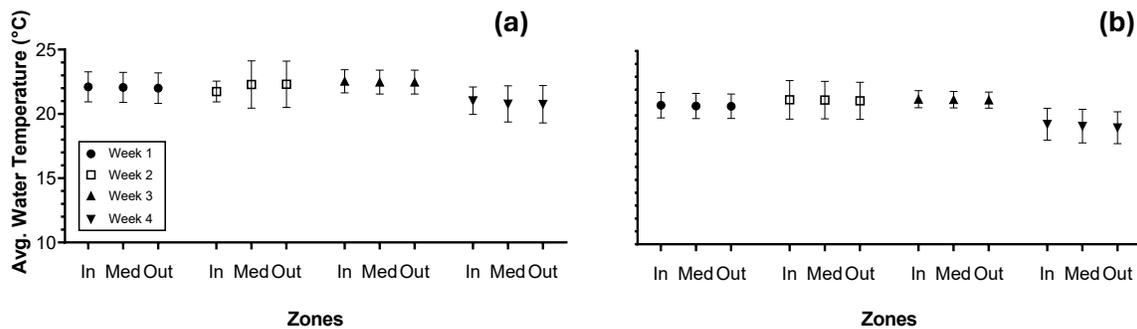


Figure 9. Temperature distribution evolution in the water, analyzed by zones in summer, for CWF (a) and PWF (b).

A similar trend was observed in autumn, although the fluctuations were smaller due to seasonality, where during the first week of the crop for CWF (Fig. 10a), PWF (Fig. 10b) and TWF (Fig. 10c), the conditions showed smaller differences than during the other weeks for the same water flow conditions.

Significant differences in the concentration of dissolved oxygen distribution between the beds with CWF and PWF were found for each elapsed week (Fig. 11). The effect of continuous water movement on the aeration of the hydroponic bed was

remarkable, where the oxygen demand for the first week of the crop was significantly higher than during the rest of the period, flattening out from the second week onwards. In addition, oxygen concentration in the zone closest to the water inlet was significantly higher than in the middle and outlet zones during weeks 2, 3 and 4 (Fig. 11a). No variation in dissolved oxygen in the water was observed for PWF during the first three weeks in all the areas sampled, while at the end of the crop, a break in the pattern was observed in the last week (Fig. 11b).

The fall of oxygen in the water for CWF, PWF and TWF was sustained and constant over time in autumn (Fig. 12), unlike in summer. Comparing the zonal evolution of CWF in summer versus autumn shows that the differences between the bed zones were significantly larger in autumn (Fig. 12a).

2.5.4 Nutrients

Alkalinity in summer varied from 230 to 35 (mg/L CaCO₃), decreasing constantly and steadily as the crop evolved, with no differences observed between CWF and PWF. Likewise, the concentration of nitrate in the water (mg/L NO₃⁻) showed a similar behavior to alkalinity, with a positive correlation between these two variables of 0.97 for CWF, and 0.95 for PWF, observing three important breaks at the same times for these indicators, as illustrated in Fig. 10.

Calcium concentration in water (mg/L Ca²⁺) during the first 14 days of culture showed a strong and prolonged decrement for CWF and PWF, being more pronounced for CWF. This tendency explained why the lettuce growth was faster for this condition than for PWF during this period (Fig. 13). However, after these two weeks, an increment of Calcium concentration was found for both types of inlet water flows, that originated an equal growth rate for CWF and PWF. The increment of the concentration of this macronutrient (c_i) can be explained by the reduction in the alkalinity of the water.

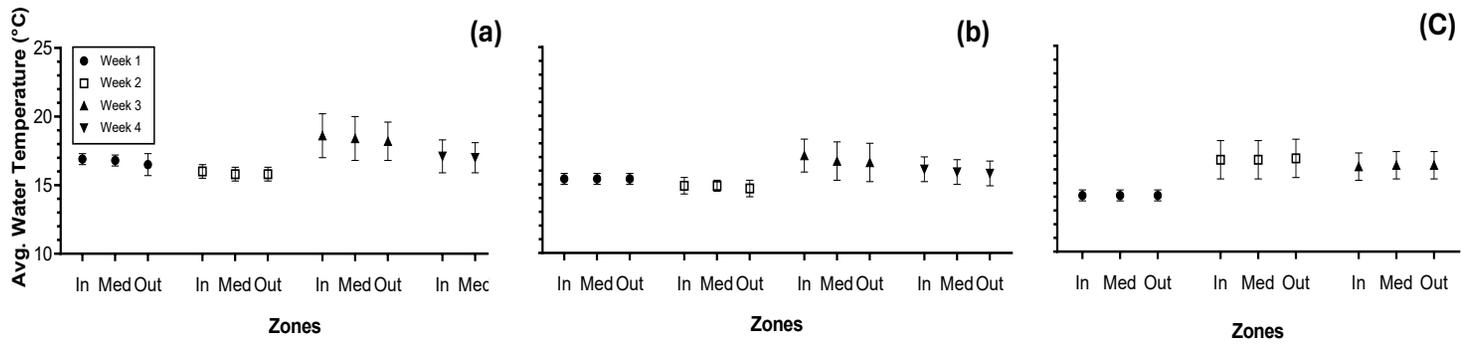


Figure 10. Temperature distribution evolution in water, analyzed by zones in autumn, for CWF (a) and PWF (b) and TWF (c).

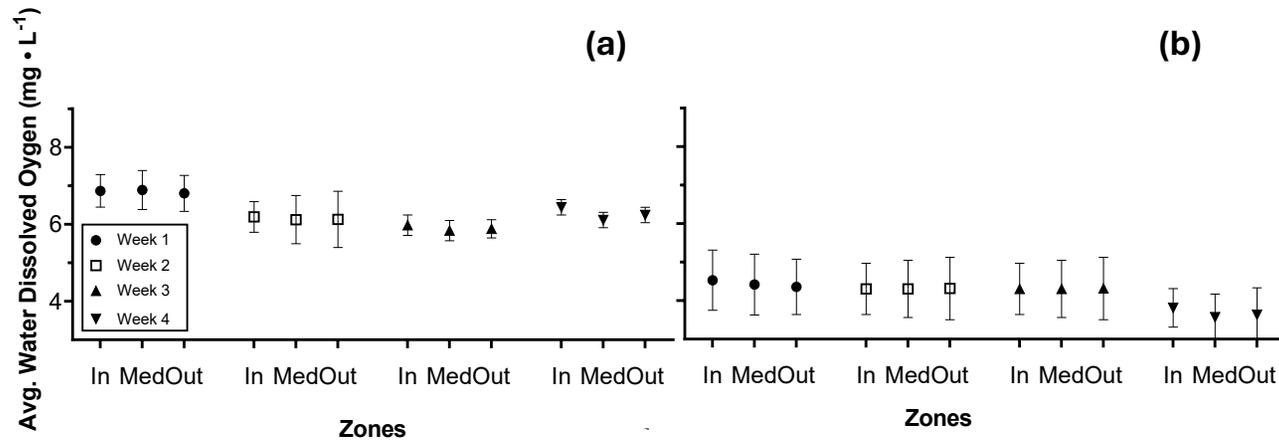


Figure 11. Average dissolved oxygen evolution in the water, analyzed by zones in summer, for CWF (a) and PWF (b).

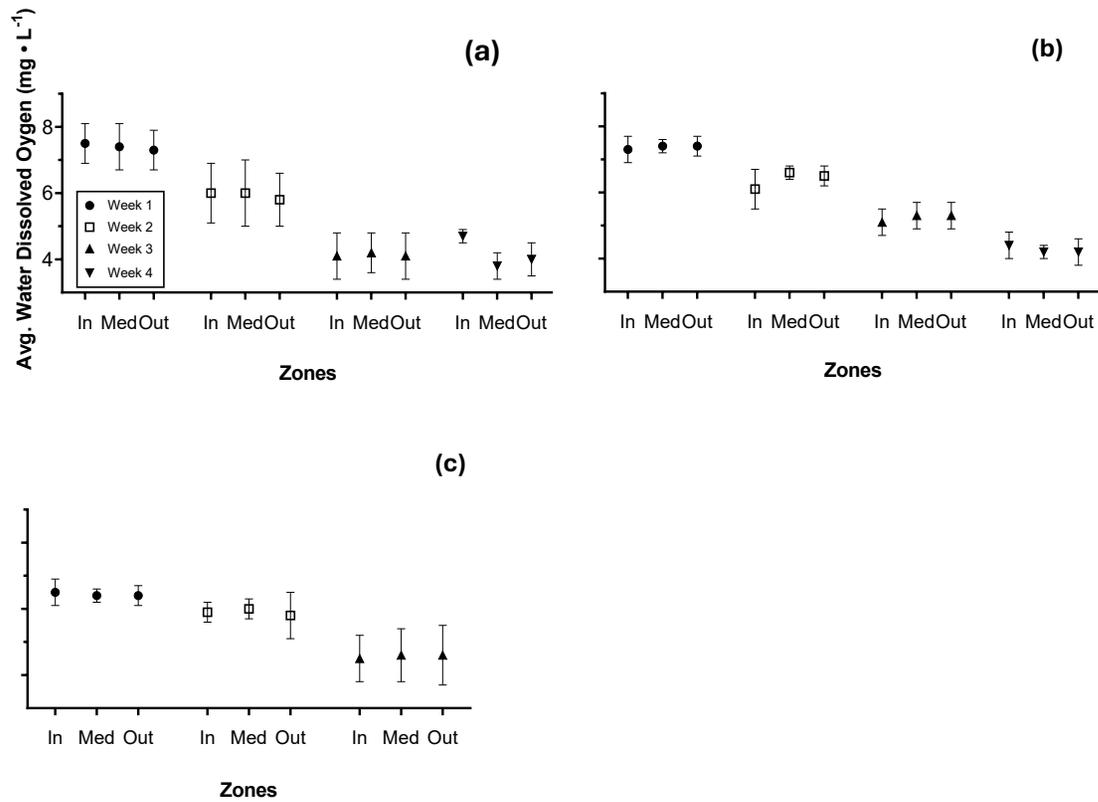


Figure 12. Average dissolved oxygen evolution in the water, analyzed by zones in autumn, for CWF (a), PWF (b) and TWF (c).

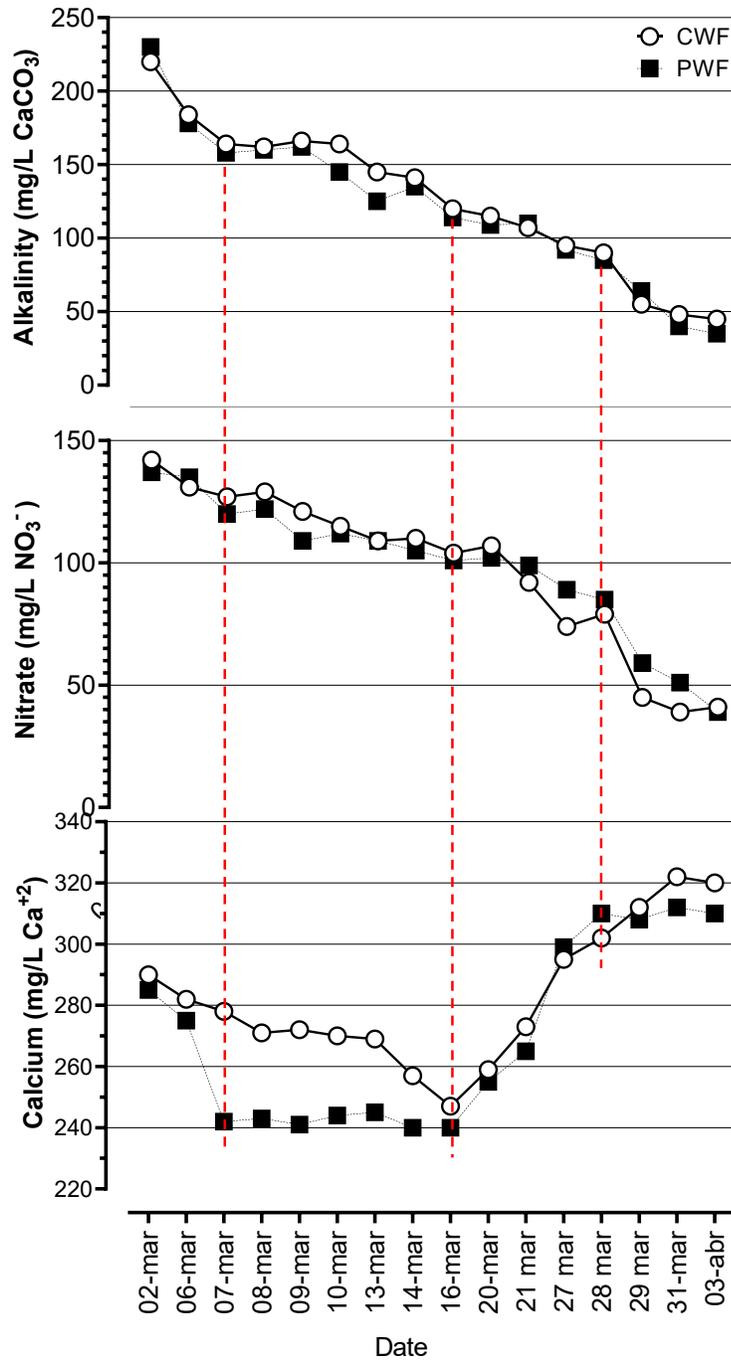


Figure 13. Evolution of Alkalinity, Nitrate and Calcium concentration in water in Summer. Red dotted lines indicate the periods of breakthrough in the tendency of these parameters.

Once the concentration c_i of CaCO_3 reached a value below 120 mg/L, the Calcium concentration began to rise, because the lettuce could no longer keep absorbing calcium in an efficient way.

In hydroponic systems, alkalinity can significantly impact calcium absorption by plants. High bicarbonate (HCO_3^-), alkalinity levels have been shown to inhibit plant growth (Pearce et al., 1999). Additionally, excessive Calcium ion (Ca^{2+}) concentrations in the irrigation water can lead to Calcium accumulation in the nutrient solution, negatively affecting the nutrient uptake and plant growth (De Freitas et al., 2006). Furthermore, the presence of Calcium and bicarbonate ions, along the variation of the pH levels, has been reported to influence iron stress mechanisms in crops (Ropokis et al., 2021).

The dynamics of alkalinity, nitrate and calcium in autumn were like those found in summer for the CWF and PWF flows, where during the first ten days there was a drop in the nitrogen concentration in the water followed by a constant rise until day 18 of crop (Fig. 14). The TWF condition showed greater stability for these dynamics. Calcium concentration, as in summer, showed an increment after the first 15 days of growth, in response to the alkalinity dynamics, which varied between 163 and 10 (mg/L CaCO_3) for the three types of inflow water.

In hydroponics, the alkalinity levels play a crucial role in the absorption of nutrients by plants. A decrease in alkalinity can have a substantial impact on the ability of plants to take up essential nutrients (Trientini et al., 2023). Lowering the pH of the nutrient solution may result in a decreased nutrient uptake by plants, ultimately affecting their growth and overall health (Gillespie et al., 2021). Literature results indicate that as alkalinity decreases, the concentrations of vital nutrients such as phosphorus, calcium, magnesium, manganese, and zinc in plant tissues also decrease (Anderson et al., 2017).

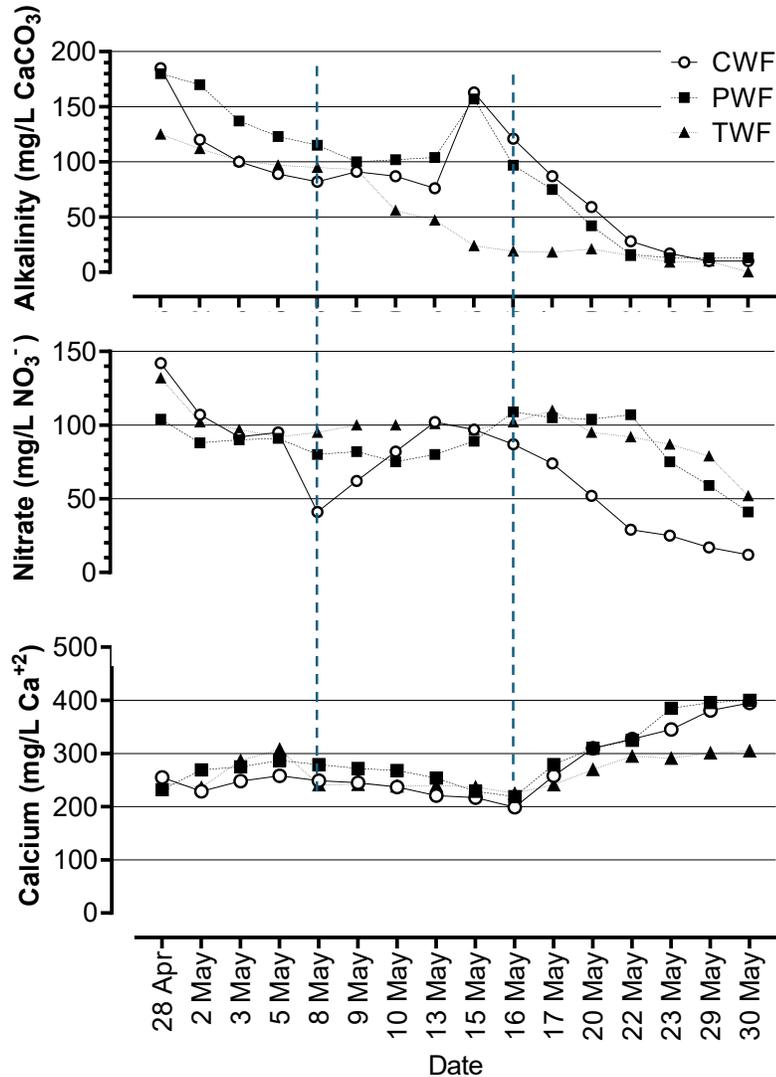


Figure 14. Evolution of Alkalinity, Nitrate and Calcium concentration in water in Autumn, blue dotted lines indicate the periods of breakthrough in the tendency of these indicators in Autumn.

Maintaining optimal alkalinity levels is essential for ensuring proper nutrient uptake by plants in hydroponic systems. When the pH of the nutrient solution is not within the appropriate range, plants may struggle to absorb essential nutrients effectively (Gillespie et al., 2020).

2.5.5 Water Dissolved Oxygen

Dissolved oxygen in the water exhibited a pH-dependence during the summer and autumn experiments, in which pH and dissolved oxygen contents depended on the average water temperature, as described in Figures 13 and 14, by a red dotted line for summer and blue for autumn. The results indicated that when water temperature was higher, the dissolved oxygen and pH contents dropped.

2.5.6 Fractional Green Canopy Cover (FGCC)

During summer and autumn seasons, sinusoidal behavior was observed for the FGCC, with a slower velocity in summer, requiring 33 days to reach 100% coverage, while in autumn was 21% faster and took 26 days. This significant difference was due to the effect of ambient temperature on water temperature, that was higher in summer causing an increased stress and a lower comfort level of the DWC culture.

2.5.7 Leaf and root growth

An average weekly leaf growth in summer was obtained for the CWF condition with a development that took between 10 to 25 mm/week, that increased for the PWF inlet water flow to values between 15 and 25 mm/week on the same period (Fig. 14), with the highest growth rates observed during the last week of cultivation.

In summer (Fig. 15), the effect of the low water velocity close to the lateral bed walls was noticed more pronounced, with clear variability in growth between the columns, particularly between column 1 and column 5. Plants closer to the bed lateral walls exhibited a better growth, as reflected by larger average leaf lengths, while those of the central area show reduced growth, likely due to nutrient depletion and higher water temperatures as the solution moves through the bed. This uneven growth emphasizes the spatial variability exacerbated by environmental conditions such as the elevated temperatures in summer.

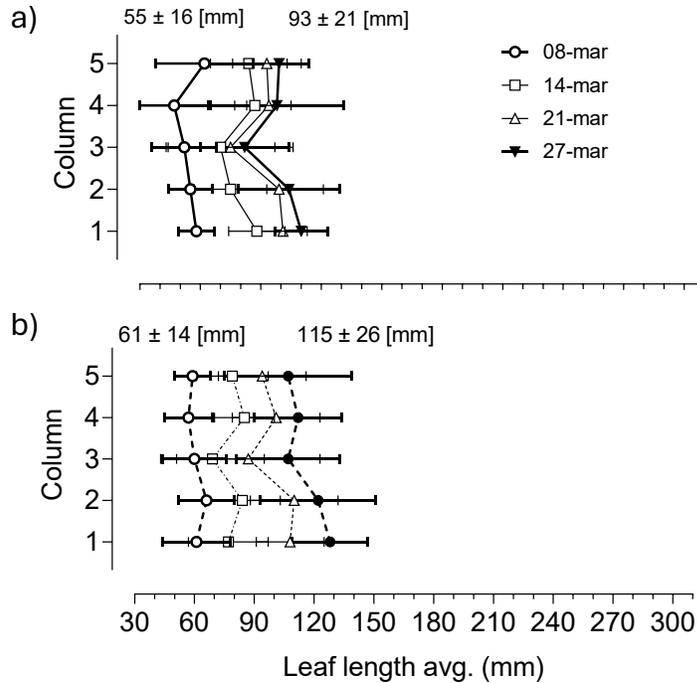


Figure 15. Spatial variability of lettuce growth across hydroponic beds, in Summer, at the zones described in Figure 11, showing weekly growth for CWF (a) and PWF (b).

The same trend of accelerated growth in the last weeks was observed in autumn, but with higher values. In this season, a growth rate that ranged from 10 to 40 mm/week was obtained for CWF, and between 40 to 150 mm/week for PWF on the same period. The experimental results indicated that lettuce growth rate was considerably higher in the TWF inlet water flow hydraulic configuration, with a leaf length that varied between 59 and 269 mm (Fig. 16), from the first to the fourth week.

The growth results obtained can be explained by a combination of a seasonal cool climate, that kept water in the bed at a lower temperature, and the use of a larger number of water inlet points (TWF), which with three water inlets favored the fluid motion and the transport of the nutrients to the lettuce roots by this successful combination that generated the fastest lettuce leaf growth and FGCC.

During autumn (Fig. 16), the edge effect at the lateral bed walls was considerably less pronounced than in Summer. The lettuce growth across all columns was

uniform, without significant differences found, as indicated by the smaller variations in the average leaf length between columns. Cooler and more uniform temperatures in autumn contributed to a better nutrient retention and to achieve a consistent water quality throughout the bed, reducing the disparity between the sizes of the plants.

Variations in temperature levels directly influenced plant growth and their ability to adapt to the environmental dynamics. By monitoring and controlling the temperature within greenhouses, growers can create and control the ideal conditions for crops to thrive, ensuring maximum productivity and quality of yield.

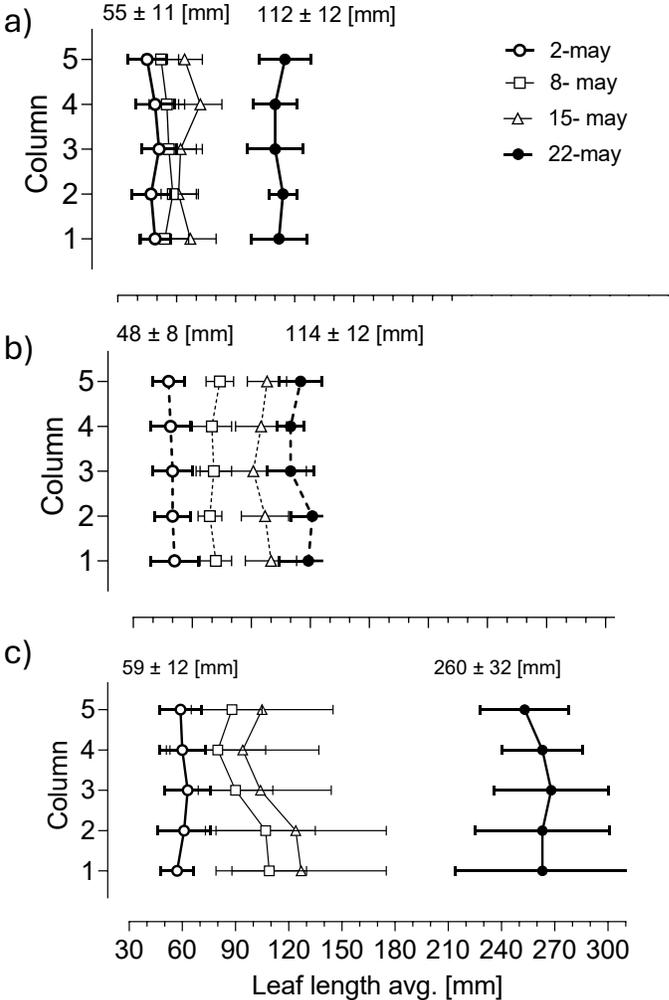


Figure 16. Spatial variability of lettuce growth across hydroponic beds, in Autumn, at the zones described in figure 1, with a weekly growth for CWF (a), PWF (b) and TWF (c).

This meticulous attention to temperature management is essential for sustainable and efficient agricultural practices. A comprehensive grasp of the relationship between climatic data and temperature fluctuations is essential for enhancing crop productivity in greenhouses. Through the utilization of data-driven insights, researchers and growers can formulate efficient climate control strategies to maintain optimal conditions for plant development in greenhouses (Shah et al., 2024).

The maintenance of suitable temperature levels in water plays a critical role in ensuring steady and high-quality crop yields, underscoring the significance of temperature management in contemporary agricultural methods (Seo *et al.*, 2021). Monitoring climatic patterns plays a vital role in comprehending temperature gradients within greenhouses and enhancing crop growth. Through the analysis of climatic data, researchers can evaluate the impact of temperature fluctuations on crop yield and develop well-informed strategies for climate regulation (Wang et al., 2023; Zhang et al., 2022).

2.6 Preliminary conclusions

This study analyzed the effects of water quality along location, type and number of water inlets in the hydroponic beds of a recirculating flow system to achieve a sustainable lettuce growth in summer and autumn at the southern hemisphere. The experimental design considered first a single water inlet in summer, either a continuous water flow (CWF) or a pulsatile water flow (PWF), with a better yield performance found for the PWF mode. Therefore, in autumn an additional three water inlet pulsatile flow (TWF) was evaluated.

Hydroponics lettuce growth is recognized as a sustainable agricultural approach in the global warming scenery. This study in a greenhouse condition revealed the relevant role of location, type, and number of water inlets in the hydroponic beds of a recirculating flow system on achieving a lettuce sustainable growth.

In summer, the use of the Pulsatile Water Flow (PWF) inlet condition, for the 20 days of crop, allowed to achieve a 15% higher growth than with the Continuous Water Flow inlet for the average leaf height indicator. In autumn, for the same growing period, the Three Pulsatile Water Flow (TWF) variant allowed to obtain an improvement of 220% of leaf height by the pulsed flow condition at three inlet points.

By implementing the TWF flow system, a growth performance of twice the magnitude for the same period and number of days was achieved in autumn. Pulsatile Water Flow with one inlet (PWF) or with three inlets TWF achieved a better lettuce growth performance for leaf cover (FGCC) and leaf height.

During summer and autumn, it was observed that the mean difference in air temperature between the outside and inside of the greenhouse in summer was approximately twice that in autumn (7.5 ± 2.9 °C compared to 3.5 ± 1.2 °C). This thermal variability influenced the quality of the water in the hydroponic beds; the water in the beds had a buffering role for the thermal variability of the air. Because of this a direct relationship was observed between water temperature and dissolved oxygen concentration as well as pH; as dissolved oxygen decreased, pH also tended to fall. This strong correlation, between -0.91 to -0.95 in Summer for CWF and PWF respectively, and from -0.95 to -0.93 when comparing the same flows and the TWF condition in Autumn, proved to be a limiting factor for optimal lettuce growth, specifically in terms of the leaf coverage of the culture. These trends remained consistent over time for the three types of water inlet flow investigated: CWF, PWF, and TWF flows.

In this experiment, beyond the previously noted benefits of pulsed flow conditions on lettuce growth (PWF and TWF), a significant distinction was observed when comparing pulsed flow water (PWF) with continuous flow water (CWF) using the same 40 W pump. Implementing pulsed pumping resulted in a reduction of the electricity consumption by up to 50 times per crop cycle compared to continuous pumping. This substantial decrease in electrical energy consumption not only lowers operational costs but also enhances the sustainability of hydroponic farming practices.

Currently, these pulsed flow modalities with three water inlets (TWF) have been implemented at the Agricultural Research Institute (INIA, Chile), achieving remarkable results in growth, allowing to shorten the crop time, and improving the possibility of obtaining economic benefits by the users of rural family farming (RFF) when implementing these technologies.

CHAPTER 3

EFFECT OF HYDRAULIC CONFIGURATION ON LETTUCE GROWTH IN HYDROPONIC BED: EXPERIMENTS AND NUMERICAL SIMULATIONS

3.1 Abstract

Experiments and computational modeling were developed to determine the effect of different types of hydraulic configurations on water quality variables to improve growth of lettuce in hydroponic beds. The variants in the hydraulic configurations consider water recirculation in hydroponic modules using Deep Water Culture technique (DWC), for continuous (CWF) and pulsatile water flow (PWF) using either one or three water flow inlets (TWF). These data were used to generate fluid mechanics and heat transfer models for the described hydraulic configurations to assess the effect of the hydraulic configuration on lettuce growth. The results obtained from the mathematical model by the finite volume method allowed to explain the influence of water flow and temperature on the rate of growing for lettuce during summer and autumn in the southern hemisphere. The main findings obtained from the hybrid numerical – experimental model to achieve high lettuce yield were that the number of water inlets influenced nutrient transport and water quality variation, where the variant with three water inlets (TWF), and the climatic condition for autumn achieve better plant growth performance than summer. Computational modelling of fluid mechanics and heat transfer allowed to predict the variation of water quality variables in DWC bed, being a suitable technique with a high potential for achieving new accurate agriculture standards.

3.2 Literature review

Globally, in the last five the harvested area of lettuce and chicory has remained around an average value of 1,213,000 ha. During the last decade the production of lettuce and chicory has been growing at an average annual rate of 2.87%, reaching 27,011,747 tons in 2021 (FAOSTAT, 2022).

The hydroponic farming approach is considered as a sustainable and viable option for minimizing water usage compared to traditional techniques. Particularly, due to the climate change current scenery, many regions are suffering from soil degradation and scarce water resources (Velazquez-Gonzalez et al., 2022), and hence horticultural hydroponic farming offers significant advantages. Notably, this farming technique is environmentally friendly, as it does not rely on pesticides and utilizes a significantly reduced quantity of water (Casey et al., 2022). Conventional farming, on the other hand, faces several challenges, mainly related to soil quality (Kannan et al., 2022) and climate (Holmes et al., 2019).

There are several alternative horticultural farming systems besides hydroponics, such as aquaponics (combining aquaculture and agriculture) and aeroponics (utilizing a mist environment for plant growth), along to the substrate culture like cultivating crops with fungi (Frasetya et al., 2021). Nonetheless, hydroponic farming has gained a significant attention due to its exceptional nutrient management and high yields (Pineda-Pineda et al., 2017).

Hydroponics is generally developed in rectangular beds, called raceways in aquaculture, using the DWC (Deep Water Culture) technique, or NFT (Nutrient Film Technique). DWC hydroponic systems are a type of stationary system that utilize floating rafts to support plants (Kozai et al., 2022). These rafts floats on the hydroponic beds which are filled with nutrients-rich water. On the other hand, NFT hydroponic systems consist of grow channels that circulate a layer of nutrient solution using a mechanical pump, without the use of a substrate (Abbasi et al., 2021). In both DWC and NFT, the influent water flows towards the effluent section located at the opposite end, and the hydraulic pattern is described as a plug flow (Lennard and Leonard, 2006; Tyson, 2005).

One of the main obstacles for horticultural hydroponic production with DWF or NFT is to maintain controlled environments in optimal conditions for the growing species, such as greenhouses and indoor modules, promising a new era of crop management (Rouphael et al., 2018). They enable a year-round production and allow the

meticulous fine-tuning of chemical compositions and bioactive profiles in vegetable crops (Ertani et al., 2021; Gumisiriza et al., 2023; Stutte, 2006).

In hydroponics, besides the aerial environment control, a keen understanding of root-zone water quality should play a key role to improve growth crop. For instance, water temperature has proved being advantageous for fast-growing leaf vegetables but can compromise the quality of fruit vegetables (i.e. tomato) (Verdoliva et al., 2021). Moreover, colored shading nets present an innovative solution for optimizing lettuce quality, reducing nitrate content, and boosting phenolic content and antioxidant activity (Sublett et al., 2018). In parallel, environmental factors exert their influence, with controlled settings delivering significantly higher crop yields compared to open-air environments (Goh et al., 2023). The growing environment emerges as a vital determinant of crop productivity. Light and nutrient management take a center stage. Higher light intensity and optimal nitrogen levels are identified as key factors in enhancing photosynthesis, yield, and crop quality. The interplay between light cycles, leaf area, and shape provides invaluable insights into lighting strategies that optimize crop growth (Gargaro et al., 2023; Taylor et al., 2006).

In the search of resource efficiency, Muharomah et al. (2020) and Yan et al. (2019) explored into water productivity and microclimatic influences, which affects the lettuce crop. Understanding the linear relationship between water use and lettuce yield, as highlighted by Sublett et al. (2018), becomes paramount in optimizing water management strategies. Meanwhile, insights from Frasetya et al. (2021) underscore the significance of microclimatic disparities inside and outside plant houses, further demonstrating the complex interplay of environmental variables.

In the field of fluid mechanics modelling-to describe water flow trajectory and nutrient concentration inside rectangular water tanks or hydroponic beds (Javier Martínez-Solano et al., 2010) mentions the effectiveness of using computational fluid dynamics (CFD), specifically by the FLUENT® software. Models for rectangular tanks which are also called plug flow reactor (PFR), such as the one developed in this work, have been studied, as well as their configurations (Mayer et al., 2023; Yan et al., 2020). A summary with previous results is presented in Table 1.

The literature review reveals the existence of a knowledge gap between experimental results on hydroponic lettuce crops and their relationship with mathematical modeling and computational simulation of the fluid mechanics and heat transfer related to the water motion in hydroponic DWC. Therefore, this study proposes the use of a novel hybrid experimental-mathematical model to predict, by numerical simulations, the effect of the water pattern flow (continuous and pulsatile), hydraulic configuration (number of inlets), and the effect of climatic season in the water velocity distribution and temperature in the hydroponic beds used for DWC. Experimental results of lettuce grown in a greenhouse hydroponic DWC system, inside a greenhouse, are used as inputs for the computational model and to validate the results that relate the spatial velocity distribution, streamlines and temperature of the water along with the lettuce growing process. A summary with previous results is presented in Table 2.

The objective was to develop and test a hydraulic model for water circulation with different flow inlets in hydroponic beds under greenhouse conditions for growing lettuces, and to perform a predictive analysis of yield and fraction green cover (FGCC). The work aimed to explain the sources of differences in growth and water quality observed in the crop, addressing gaps in knowledge specifically for the DWC technique, providing quantitative descriptions of the seasonality effect on growth and how this source of variability would affect the distribution of variables in the water and influence growth.

3.3 Goal of the study

To determine how different hydraulic configurations in Deep Water Culture (DWC) hydroponic beds influence water quality variables and, consequently, the growth performance of lettuce, through the integration of experimental trials and computational modeling of fluid mechanics and heat transfer.

Table 2. Summary with relevant studies in hydroponics.

Authors	Description	Main findings	Limitations
Gargaro et al., 2023	This study investigates the factors influencing lettuce yields in Controlled-Environment Agriculture (CEA) systems, comparing CEA with field-based agriculture. Additionally, the study conducts a meta-analysis on lettuce growth in CEA systems to gain insights into yield improvements.	<ul style="list-style-type: none"> • CEA yields are double that of field-based agriculture, with 3.68 kg/m². • Vertical farming increases lettuce yield to 6.88 kg/m². • Variety, season, lighting, and nutrient delivery influence lettuce yield. • CEA lettuce grows faster, 50x in summer and 300x in winter. 	<ul style="list-style-type: none"> • Incomplete report of the key trial data by published studies.
Casey et al., 2022	This research compares the environmental footprints of hydroponic lettuce with field-based supply, focusing on environmental impacts, energy use, and the sustainability of lettuce production.	<ul style="list-style-type: none"> • Lettuce footprints vary significantly based on energy sources and systems. • Closed hydroponic systems can mitigate water stress and soil degradation. 	<ul style="list-style-type: none"> • Lack of data on greenhouse systems using natural lighting. • Absence of nutrient balance for periodic maintenance in field systems.
Kannan et al., 2022	This paper provides a state-of-the-art review of hydroponics worldwide, highlighting the current advancements and	<ul style="list-style-type: none"> • Hydroponic farming minimizes water wastage by supplying water directly to plant roots, reducing evaporation, and 	<ul style="list-style-type: none"> • Higher initial land cost for hydroponic farming. • Risk of water-borne diseases due to shared nutrient solution.

	practices in hydroponic farming.	allowing reuse of nutrient solutions.	<ul style="list-style-type: none"> ● Warm climate and inadequate oxygenation can limit yield production.
Gumisiriza et al., 2022	The study assesses lettuce growth using non-circulating hydroponics in Uganda, compared to traditional soil-based cultivation.	<ul style="list-style-type: none"> ● At harvest time, there was no significant difference in plant height, number of leaves, and leaf width, but a significant difference existed in dry matter content between the two farming systems. 	<ul style="list-style-type: none"> ● The study was conducted under non-controlled environmental conditions, which could introduce variability in the results and limits the ability to draw definitive conclusions.
Frasetya et al., 2021	This research evaluates various hydroponic systems to enhance the efficiency of lettuce growth, comparing different techniques and their outcomes.	<ul style="list-style-type: none"> ● NFT system is 6-10% more efficient than RFS and DFT systems. ● NFT and RFS systems are recommended for hydroponic lettuce production. 	<ul style="list-style-type: none"> ● Water shortages inhibit growth and reduce crop yields. ● Components must be available proportionally to avoid growth inhibition.
Javier Martínez-Solano et al., 2010	This study models flow and concentration in rectangular water tanks using CFD, focusing on the impact of water quality in storage tanks and distribution systems.	<ul style="list-style-type: none"> ● 82% of tank volume under complete mixing conditions. ● Velocity and concentration gradients showed good correlation in the tank. 	<ul style="list-style-type: none"> ● Complete mixing assumption not suitable for all tank configurations. ● Some models require a priori assumptions that were not verified.
Mayer et al., 2023	The research focuses on scale-up challenges in biotechnological production	<ul style="list-style-type: none"> ● Biomass yield reduced by 11%, and the specific product yield by 20%. 	<ul style="list-style-type: none"> ● Validation of computational results is

	and utilizes CFD simulations to design and characterize a scale-down reactor.	<ul style="list-style-type: none"> ● Flexibility of scale-down setup with CFD simulations for scale effects. 	challenging due to sensor limitations.
Dutta et al., 2023	Hydroponics enhances crop productivity and quality by using optimal pH and electrical conductivity (EC) levels. Discusses the benefits of hydroponics, including improved growth rates and crop quality, various growth media, different techniques used, and the potential advantages of this agricultural method.	<ul style="list-style-type: none"> ● Hydroponics enhances crop productivity and quality. Training programs can promote hydroponic techniques among farmers. Hydroponics can play a vital role in future sustainable agriculture. 	<ul style="list-style-type: none"> ● Maintaining optimal EC, pH, and TDS values for growth in hydroponics is challenging. Additionally, there is a lack of information on IoT-enabled automated systems for hydroponics.
Karunakaran et al., 2022	The study investigated the use of IoT technology in hydroponic systems for growing tomatoes. Sensors connected to an Arduino system were used to monitor temperature, humidity, pH and electrical conductivity. Tomatoes were grown in a mixture of coco coir and	<ul style="list-style-type: none"> ● The study showed a 34-67% improvement in key quality characteristics of seedlings grown in the hydroponic system compared to soil. The system was water and nutrient efficient, and sensor data was collected in real time and stored in the cloud. Solar panels proved to be a viable energy source for the system. 	<ul style="list-style-type: none"> ● The main limitations include the need for a reliable power source, partially addressed by solar panels, and the upfront costs of investing in sensors and IoT equipment. In addition, the system relies on technical expertise for setup and maintenance,

perlite, and compared to plants grown in traditional soil.

Aires et al.,
2023

The study optimized a hydroponic lettuce system to enhance nitrogen (N) and phosphorus (P) removal efficiency, achieving high biomass production while reusing wastewater for irrigation. It successfully managed low N and P levels in wastewater, ensuring minimal eutrophication, and achieved significant N and P removal.

- The study found that total biomass was similar in all treatments, with high nitrogen (N) and phosphorus (P) removal. A daily light integral (DLI) of 10-12 mol m⁻² d⁻¹ was optimal for lettuce growth. Additionally, the hydroponic wastewater met the N and P criteria for reuse in irrigation.

which can be a barrier for some farmers.

- The study highlighted that uneven light distribution due to artificial light limitations affected nutrient removal efficiency. Variations in nutrient removal were observed based on different light and nutrient levels.

3.4 Methodology

The methodology employed in this research included two main stages: (1) controlled lettuce growing experiments in three hydroponic beds under different inlet water configurations, and (2) the mathematical modelling of the fluid mechanics and heat transfer inside the beds along the numerical simulations to predict the effect of water velocity and temperature by the finite volume method in the crop growing.

3.4.1 Experimental procedure

The lettuce growth experiments, as illustrated in Figure 17, were carried out in a 177 m³ experimental greenhouse facility, constructed by a three-layers polyethylene, 130 microns thick with two-season UV additive and 89% light transparency, and deployed in the facilities of INIA Intihuasi, La Serena, Chile (-29° 91'90" S, -71° 24'84" W). The relevant parameters: air temperature ($\pm 0.1^{\circ}\text{C}$), solar radiation ($\pm 1 \text{ W/m}^2$) and relative humidity ($\pm 0.1\%$) inside the greenhouse (Fig 17), as well as the air temperature outside the greenhouse ($\pm 0.1^{\circ}\text{C}$) were measured automatically each 15 minutes, using a Sensor ATMOS 14, and then stored in a datalogger.

Solar radiation inside the greenhouse, and air temperature inside and outside the greenhouse were measured with a meteorological station sensor ATMOS 14. Two sets of unique growth experiments were accomplished in summer and autumn in the southern hemisphere of 2023.

The experiences in summer lasted 33 days, from March 1st to April 3rd, 2023, in which the minimum external air temperature was 12.8°C, the maximum one of 22.6 °C, and the average temperature 17.2°C, while inside the greenhouse the values of the air temperature was $25.9 \pm 3.2^{\circ}\text{C}$. In autumn the lettuce growth required 31 days, beginning in April 22 and ending on May 23, 2023.

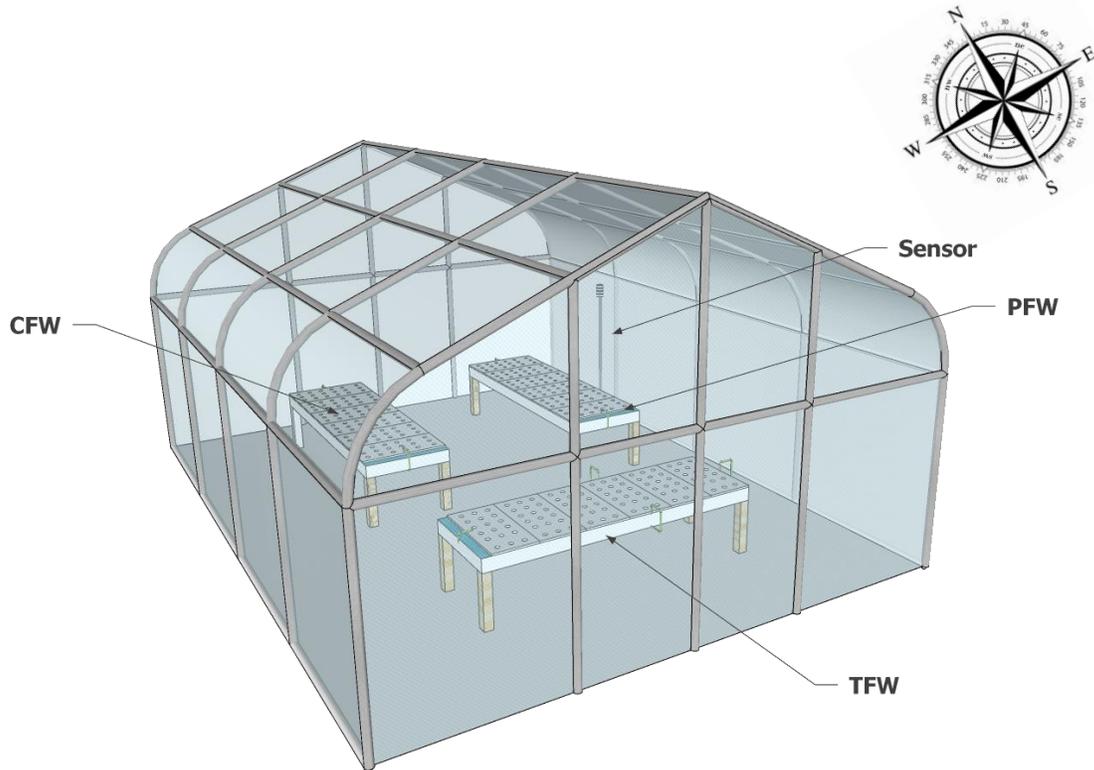


Fig. 17. Greenhouse, sensors, and the three water beds used in the experiments: CFW: one inlet continuous water flow; PFW: one inlet pulsatile, and TFW: three pulsatile water inlets.

The values of the air outside the greenhouse were a minimum of 8.72 °C, maximum of 22.2 °C, with an average value of 13.9 °C.

The average solar radiation inside the greenhouse for the summer season varied between 0 and 340 W/m², while in autumn the variation was between 0 up to 178 W/m². The best yields, in terms of lettuce size and weight, were obtained in the autumn season in the southern hemisphere.

During summer, the growth of lettuce in hydroponics Deep Water Culture (DWC) was studied by physical experiments assisted by mathematical modeling and numerical simulations. The capacity of each of the three hydroponic beds was 300 liters of water (300 x 100 x 10 cm). Each hydroponic bed was built in wood, lined on the inside by a plastic of 4 mm in thickness. A 0.04 HP washing machine pump,

located in the background, and outside of each hydroponic bed, recirculated the water in each bed, through a unique influent and effluent. Figure 18 depicts the experimental flow setup, and the instruments used to measure ambient temperature, radiation, and relative humidity inside the greenhouse.

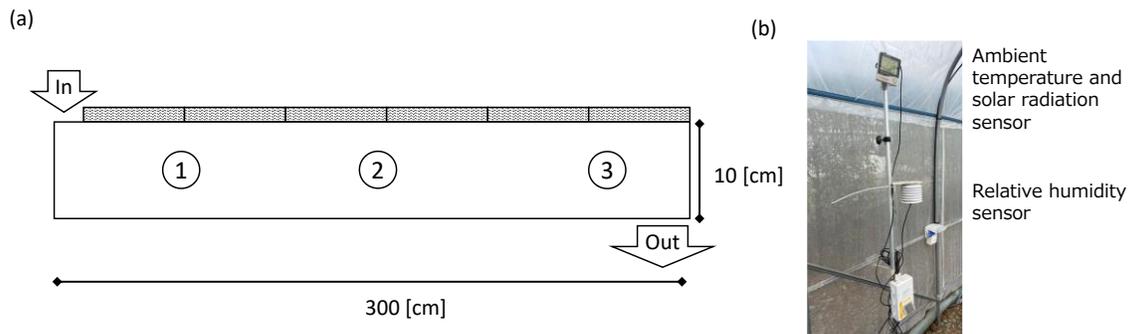


Fig. 18. Experimental setup: (a) The dimensions of the hydroponic bed with a width of 110 cm are indicated; (b) Remote weather station implemented for the measurement of ambient temperature, solar radiation, and relative air humidity inside the greenhouse.

Each one of the hydroponic beds was dimensioned to install up to six polystyrene foam boards (50 x 100 x 3 cm) on the water surface. The platters were perforated to have each one a planting frame of 15 lettuce seedlings, held in sponge, totaling 90 plants in each hydroponic bed, as shown in Figure 19, along the water inlet flows. The first hydraulic condition investigated on hydroponic lettuce growth was the effect of the type of water flow at the inlet in three hydroponic beds. The first one was the base case with a Continuous Water Flow (CWF). The second water inlet design considered an identical hydroponic bed fed by a Pulsatile Water Flow (PWF). Finally, the third hydroponic bed had the same conditions as the PWF, but here water was entering through three inlets (TWF), one in the axial direction, at the center of the left surface, and the other two in the lateral surfaces, near the entrance (Fig. 19).

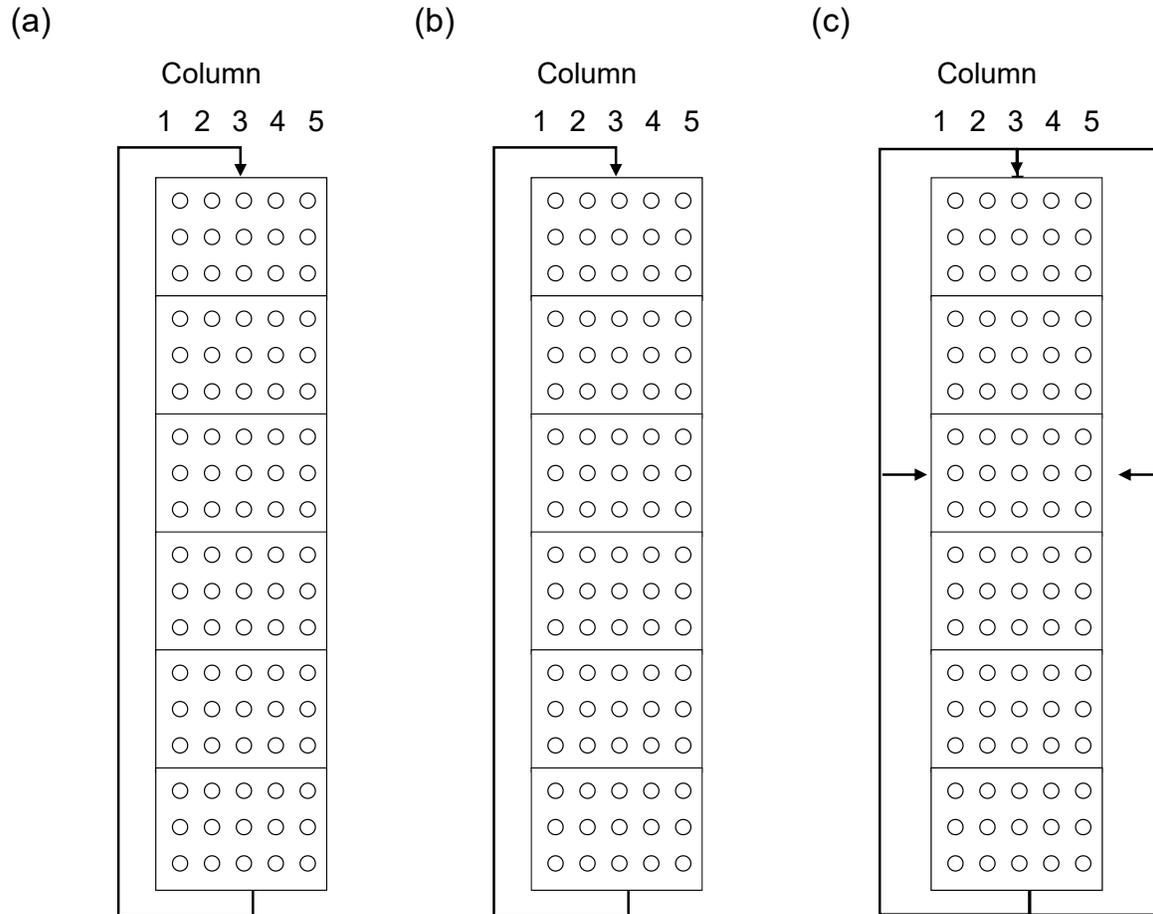


Fig. 19. Lettuce and inlet water arrays used in the experiments. Lettuces samples were taken from the hydroponic boards hole rows where indicators of leaf growth and leaf/root ratio along the hydroponic bed were averaged for: (a) CWF at continuous flow, (b) PWF at pulsatile flow and (c) TWF operated by three inlets pulsating flow.

The hydroponic bed identified as CWF had a recirculating continuous flow, through a plastic hose with an inner diameter of $\frac{1}{4}$ ", of water with 0.17 L/min, for 24 h/d during all the lettuce growth period, while the PWF operated in 20-minute pulses of water, every 2 hours, 6 times a day. The water velocity was estimated by measuring the volumetric flow rate. Table 3 displays the values of the inlet water velocity, flow duration and the intervals of time of operation.

Table 3. Hydraulic inlet water configurations for summer and fall seasons.

Flow type	Inlet velocity [m/s]	Flow duration [h/d]	Interval on
Continuous Water Flow (CWF)	0.004	24	Permanent
Pulsatile Water Flow (PWF)	0.004	2	20 minutes
Three Pulsatile Water Flow (TWF)	0.004	2	20 minutes

3.4.2 Experiment design

During lettuce growing, three water quality parameters: water temperature ($^{\circ}\text{C}$), pH and dissolved oxygen ($\text{mg O}_2/\text{L}$), were measured daily within each hydroponic bed, at three zones, mid-water, of the third column (Fig. 18a), by submerging a multiparameter electrode attached to a Hanna HI2400 portable instrument.

The nutrient solution was based in a Steiner formulation (1961), who established the relationship between NO_3^- , PO_4^- and SO_4^{2-} anions and K^+ , Ca^{2+} and Mg^{2+} cations. The details of the amounts and proportions of each nutrient used in the experiments are described in Table 4.

Table 4. Nutrient solution added to water in the lettuce hydroponic beds.

	Product	Quantity
Nutrient Solution A	Ultrasol desarrollo TM *	500 [g]
	Potassium sulfate	100 [g]
	Phosphoric acid 80%	43 [cc]
	Magnesium sulphate	50 [g]
	Iron Chelate	17 [g]
Nutrient Solution B	Calcium nitrate	450 [g]
	Potassium nitrate	500 [g]

* Ultrasol Development TM is a soluble crystallized NPK fertilizer, containing 54% w/w NH_4 , 46% w/w NO_3 , 18% w/w N and 18% K_2O .

3.4.3 Lettuce Leaf growth and Leaf/Root ratio

The transplanted lettuce seedlings of the Qunity variety, were acquired at a stage of development with four true leaves, and a total length of 31 ± 1 mm. The leaf growth and leaf/root ratio performance indicators were estimated by calculating the averaged values of the measurements obtained experimentally. The hydroponic polystyrene foam boards were divided into 5 axial rows, with row 1 and 5 being the ones close along to the walls, while row 3 was the central one, as illustrated in Figure 20.

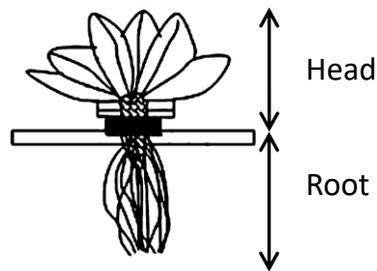


Fig. 20. Head and root measured zones of lettuce (Modified from Wang et al., 2021).

The length of the head and root of lettuce were measured according to the schematic representation provided in Fig. 20. The leaf growth of lettuce in summer independent of the number of flow inlets, was lower than in the autumn, being 93.06 ± 21.15 mm with CWF, and 112.14 ± 12.05 mm for PWF. In Autumn, with the CWF condition the growth was 115.23 ± 26.30 mm, and 114.97 ± 12.00 mm for PWF. TWF, operating in a pulsatile pattern, resulted in a significant improvement on the average leaf size that reached a value of 175 ± 18.45 mm, with an increment of 52% with respect to CWF and PWF.

3.4.4 Hydraulic and thermal prime background

Fluid mechanics and heat transfer play a key role in hydroponic growth of crops. Water flow transport nutrients to the crop roots and hence local pressure and velocity distributions inside the bed must be known during the growing process. The knowledge of water temperature is also important because temperature is one of the

relevant parameters to achieve a fast-growing crop in a short cycle to produce high quality products. To understand how heat transfer process occurs in hydroponic greenhouses, experimental studies have generally been applied, mainly due to the multiple factors that interacts in this problem, such as solar radiation, its effect on evapotranspiration and the annual and seasonal thermal gradients in which crop cycles are developed.

CFD simulation was used for Mesmoudi et al., (2017) to analyze the greenhouse climate and energy transfer process. They investigated the impact of vent arrangements and crop row orientation on ventilation. Their findings indicated that plastic greenhouse performs better in climate homogenization and thermal energy storage during the nocturnal period. Also, they found that the high absorptivity of the covering material reduces the available Photosynthetically Active Radiation (PAR). On the other hand, Taki et al., (2018) developed accurate greenhouse models for inside environment control and prediction, by investigating mathematical models for heat and mass transfer in greenhouses, concluding that solar assisted heat pump systems can meet the heating and cooling demand required.

Lebre et al., (2021) studied greenhouses structures using computational simulation to compare the computational results with measured values from a greenhouse prototype. Theirs findings were that better insulation and multiple layers of covering material reduced the heating demands, when a three-layer cover solution with an air gap of 13 mm was used, providing the highest temperature. Finally, they indicated that an outdoor air temperature difference of 16°C can cause a difference of about 10°C inside the greenhouse.

Adesanya et al., (2022) investigated the thermal performance of a glass greenhouse, using different mathematical models to calculate the radiation inside the greenhouse. This study accurately predicted the greenhouse internal temperature and heating demand. However, a factor that was not considered in these studies was the presence, growth and development of the plants in the greenhouse. These are some of the key issues that are analyzed in our study, relating the measured leaf

and root sizes in hydroponic beds with the CFD models of fluid mechanics and heat transfer.

In terms of fluid dynamic modeling in hydroponic beds, according to our literature review, the CFD tool has not been used in hydroponics. Therefore, we propose its use to understand how fluid mechanics can improve the understanding of nutrient transport in water, and how these fluid dynamic predictions can contribute to explain the heat transfer phenomena of convection and conduction, and how these in turn impact plant growth performance.

3.4.5 Mathematical model for water and heat flow in the bed

The assumptions used to build the mathematical model describing the transport phenomena related to the water and heat flows inside the lettuce hydroponic bed are indicated next. Water was considered a Newtonian-incompressible fluid, in a laminar flow regime, with constant properties, while those characterizing the heat transfer included: the forced heat convection in water, with the rate of change on the internal energy to account for heating during daytime and cooling at night, in a transient process and negligible viscous dissipation. Velocity (U, V, W), pressure (P) and temperature (θ) of water recirculating in the hydroponic beds were calculated from the conservation principles of mass, linear momentum and energy by the continuity, Navier-Stokes and energy partial differential equations.

$$\frac{\partial U}{\partial X} + \frac{\partial V}{\partial Y} + \frac{\partial W}{\partial Z} = 0 \quad (2)$$

$$\left[\frac{\partial U}{\partial \tau} + U \frac{\partial U}{\partial X} + V \frac{\partial U}{\partial Y} + W \frac{\partial U}{\partial Z} \right] = -\frac{\partial P}{\partial X} + \frac{1}{\text{Re}} \left[\frac{\partial^2 U}{\partial X^2} + \frac{\partial^2 U}{\partial Y^2} + \frac{\partial^2 U}{\partial Z^2} \right] \quad (3)$$

$$\left[\frac{\partial V}{\partial \tau} + U \frac{\partial V}{\partial X} + V \frac{\partial V}{\partial Y} + W \frac{\partial V}{\partial Z} \right] = -\frac{\partial P}{\partial Y} + \frac{1}{\text{Re}} \left[\frac{\partial^2 V}{\partial X^2} + \frac{\partial^2 V}{\partial Y^2} + \frac{\partial^2 V}{\partial Z^2} \right] \quad (4)$$

$$\left[\frac{\partial W}{\partial \tau} + U \frac{\partial W}{\partial X} + V \frac{\partial W}{\partial Y} + W \frac{\partial W}{\partial Z} \right] = -\frac{\partial P}{\partial Z} + \frac{1}{\text{Re}} \left[\frac{\partial^2 W}{\partial X^2} + \frac{\partial^2 W}{\partial Y^2} + \frac{\partial^2 W}{\partial Z^2} \right] \quad (5)$$

$$\left[\frac{\partial \theta}{\partial \tau} + U \frac{\partial \theta}{\partial X} + V \frac{\partial \theta}{\partial Y} + W \frac{\partial \theta}{\partial Z} \right] = \frac{1}{\text{Pr}} \left[\frac{\partial^2 \theta}{\partial X^2} + \frac{\partial^2 \theta}{\partial Y^2} + \frac{\partial^2 \theta}{\partial Z^2} \right] \quad (6)$$

The transport phenomena of water flow and heat transfer in the hydroponic beds were described in terms of the dimensionless dependent and independent variables defined as follows,

$$U = \frac{u}{U_0} \quad V = \frac{v}{U_0} \quad W = \frac{w}{U_0} \quad P = \frac{p}{\frac{1}{2}\rho U_0^2} \quad \theta = \frac{T - T_w}{T_f - T_w} \quad (7)$$

$$X = \frac{x}{l_x} \quad Y = \frac{y}{l_y} \quad Z = \frac{z}{l_z} \quad \tau = \frac{t}{l_y^2/\nu} \quad (8)$$

where u_0 represents the velocity inlet [m^*s^{-1}], ρ is the density of the fluid, [kg m^{-3}], l_x , l_y , l_z are the dimensions of the hydroponic bed in x, y and z coordinates. τ the dimensionless time, ν is the kinematic water viscosity, while the subindexes are f for air and w for water.

The values used in the numerical simulations for the Reynolds number ($Re = U_0 D / \nu$) and Prandtl number ($Pr = \nu / \alpha$) were equal to 200 and 6.99, respectively. Due to the value of the Reynolds number that was smaller than 2,300 the water flow was consider to be laminar in the mathematical model.

The analysis of the recirculating water flow during the lettuce stage growth, included the no-slip boundary conditions at the hydroponic bed walls and at the upper interface between water and the polystyrene foam boards supporting the lettuces. In the continuous water inlet flow condition (CWF), a parabolic velocity profile with an averaged horizontal velocity component $u = 0.014$ m/s was imposed at the hydroponic bed inlet, equivalent to a flow rate of 0.15 L/min, while at the exit the water flow was assumed to be developed.

Heat transfer in the water was analyzed during a transient process due to the changes of the air temperature inside the experimental greenhouse facility, provoked by the external variation of irradiation, time-varying wind velocity and external ambient temperature caused by seasonal changes and by the day-night cycle. Initially, the water temperature in each bed was assumed to be uniform, $T_o = 17^\circ\text{C}$.

Figure 21 depicts the boundary conditions used in the CFD included no-slip between water flow and solid walls, external thermal radiation and convective heat transfer from surrounding air to the hydroponic bed system, and conjugate heat transfer boundary conditions between different materials; at solid materials interfaces: same temperature: $T_i = T_j$, and energy conservation: $-k_i \text{grad}(T_i) = -k_j \text{grad}(T_j)$. Thermal contact resistances at the interfaces between the wood and the polyethylene were not taken into account, because the special care taken during the water beds construction to minimize and reduce the size of eventual air pockets. Typical values of TCR range from 0.001 to 0.01 $\text{m}^2\cdot\text{K}/\text{W}$ for polyethylene-water interfaces, 0.1 to 1.0 $\text{m}^2\cdot\text{K}/\text{W}$ for wood-polystyrene, and 0.05 to 0.2 $\text{m}^2\cdot\text{K}/\text{W}$ for polyethylene-polystyrene.

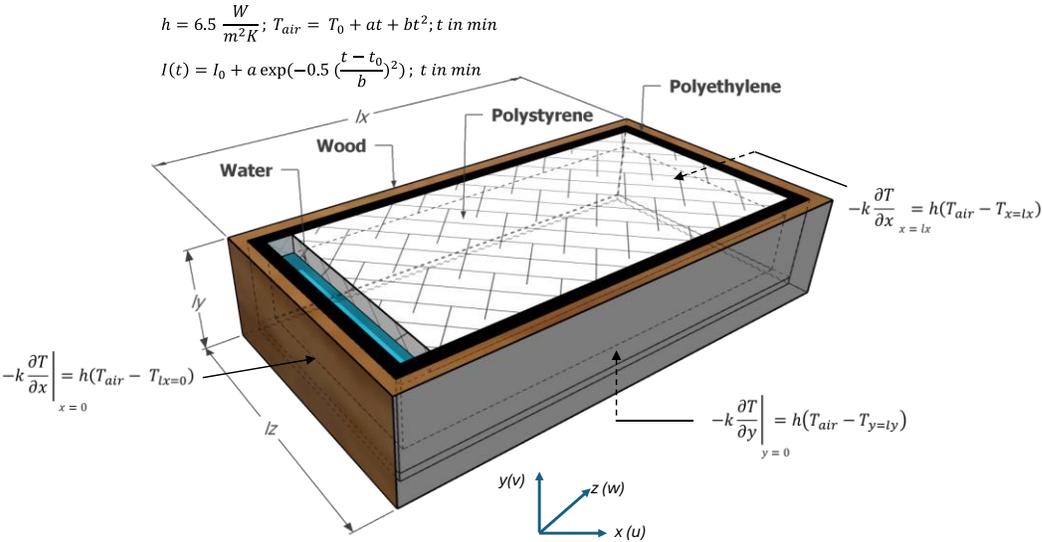


Figure 21. Thermal parameters, equations, thickness and dimensions of the materials used in the thermal model.

The information with the values of density, specific heat and thermal capacitance of water, wood and polyethylene foam used in the study are provided in Table 5. Thermal capacitance (*) was calculated as $T_c = v \mu S_h$, where V is the volume (m^3), ρ density (k/m^3), and c_p the specific heat ($J/kg \cdot K$).

Table 5. Materials, dimensions and thermal properties of the materials involved in the study.

Unit	Dimension (cm)	Volume (m^3)	Density (kg/m^3)	Specific heat ($J/kg \cdot K$)	Thermal capacitance (J/K) *
Water (Cengel & Ghajar, 2015)	3.08 x 1.03 x 0.1	0.31724	997 ^a	4,186 ^a	1,323,983
Wood (Moran & Shapiro, 2010)	2x (3.08 x 0.25 x 0.02) 2x (1.03 x 0.02 x 0.25) 1x (3.08 x 0.25 x 0.01)	0.0488	650 ^b	1,700 ^b	53,094
Expanded polystyrene (Ashby & Johnson, 2014)	6x (0.5 x 0.04)	0.12	20 ^c	1,210 ^c	2,904

3.4.6 Numerical simulations

Computational modeling was accomplished by solving the mathematical model with the finite volume method, by using the ANSYS Fluent Version 19.2. Diffusion terms, that included the viscous forces and the heat transfer by conduction in the water column, were calculated with a second order accuracy using linear interpolation functions between the nodes, for velocity and temperature. Convective terms in the Navier-Stokes and the energy equations were computed by the fifth power interpolation law. Coupling between velocity, pressure and temperature was performed by the SIMPLER prediction-correction algorithm (Patankar, 1981).

Successive under-relaxation parameters were used to calculate velocity, pressure and temperature, with values of $\alpha_v = 0.6$; $\alpha_p = 0.55$ and $\alpha_T = 0.9$. The convergence criteria to stop the iterative calculation procedure was to impose that the difference between the values calculated at two successive iterations, in each discrete finite volume and at each time step $\varepsilon_\theta \leq 10^{-5}$, for each dependent variable (water velocity and temperature). The workstation used to run the simulations had a 20-core Intel Xeon (R) CPU E5-2650v4 @2.20 GHz.

Mesh size and time step were found by solving the problems using staggered three-dimensional grids. Meshes of 520,000, 720,000 and 1,200,000 elements were selected. The verification that the results of water velocity and temperature in the lettuce beds were independent of the mesh size, mesh independence criterion, indicated that the 720,000-element mesh must be selected. The value of the horizontal component of the water velocity and that of the temperature were calculated with three mesh sizes at the central point. The values calculated with the computational model are presented in Table 6.

Table 6. Water velocity and temperature, at the central point of the hydroponic bed, calculated for the three mesh sizes tested.

Mesh size No. elements	Velocity m/s	Temperature °C
520,000	0.00120	21.51
720,000	0.00090	20.14
1,200,000	0.00088	20.14

The transient study of the water temperature evolution was carried out from May 12th up to the 17th of May, due to the significant temperature variation measured in these days. Numerical simulation of the fluid mechanics and heat transfer mathematical model for heat flow in the hydroponic bed water column was accomplished by using three values of the time steps of 60, 300 and 600 [s], after the selection of the mesh size previously described. The maximum value for the water temperature difference obtained between the three-time steps investigated was 0.41 °C.

The transient data of air temperature and radiation measured at ten minutes interval during a four consecutive days period (May 12 to May 16, 2023) were adjusted by second-order degree polynomials, in which $T_{air}(t) = T_o + at + bt^2$ ($0.85 < R^2 < 0.97$), and $I(t) = I_o + ct + dt^2$ ($0.91 < R^2 < 0.98$). The values of T_o , I_o and the four coefficients (a, b, c, and d) are given in Tables 7 and 8.

Table 7. Coefficients of the adjusted air temperature inside the greenhouse for autumn experiments (12-16 May 2023).

Days	Hour	a (°C·min ⁻¹)	b (°C·min ⁻²)	T _o (°C)	R ²
May 12	00:00 – 12:50	13,5314	13,5314	1,0454E-05	0.857
	13:00 – 23:50	26,9690	-0,0200	7,7798E-06	0.096
May 13	00:00 – 12:50	53,6285	-0,0459	1,2999E-05	0.921
	13:00 – 23:50	-10,7133	0,0329	-9,0684E-06	0.910
May 14	00:00 – 12:50	62,4143	-0,0378	6,8924E-06	0.831
	13:00 – 23:50	158,2142	-0,0658	7,5259E-06	0.769
May 15	00:00 – 12:50	420,3678	-0,1776	1,9444E-05	0.926
	13:00 – 23:50	302,1177	-0,0859	6,1417E-06	0.956
May 16	00:00 – 12:50	507,6765	-0,1719	1,4898E-05	0.956
	13:00 – 23:50	777,6371	-0,2136	1,4984E-05	0.956
May 17	00:00 – 12:50	2299,8904	-0,6105	4,0765E-05	0.944
	13:00 – 23:50	647,8211	-0,1316	6,6982E-06	0.953

Table 8. Value of the coefficients used to approach the experimental results of air radiation inside the greenhouse (12-16 May 2023).

Days	Hour	a ($W \cdot m^{-2}$)	b (min)	t_0 (min)	I_0 ($W \cdot m^{-2}$)	R^2
May 12	00:00 – 23:50	74,9779	118,3775	806,6419	0,5971	0,930 5
May 13	00:00 – 23:50	54,8995	158,4425	2260,9686	-1,5096	0,915 3
May 14	00:00 – 23:50	62,4774	119,3303	3643,9842	-0,3893	0,983 0
May 15	00:00 – 23:50	110,0374	125,3151	5114,7475	-0,0343	0,967 2
May 16	00:00 – 23:50	80,4886	146,4176	6520,9557	-1,6442	0,907 6
May 17	00:00 – 23:50	121,3072	129,7085	7968,1299	-0,7297	0,979 9

The evolution of the thermal boundary conditions from May 12th up to the 16th of May, by radiation and heat convection, using the fitted described in Tables 6 and 7, are illustrated in Figure 22, by the transient values of air temperature and radiation measured inside the greenhouse.

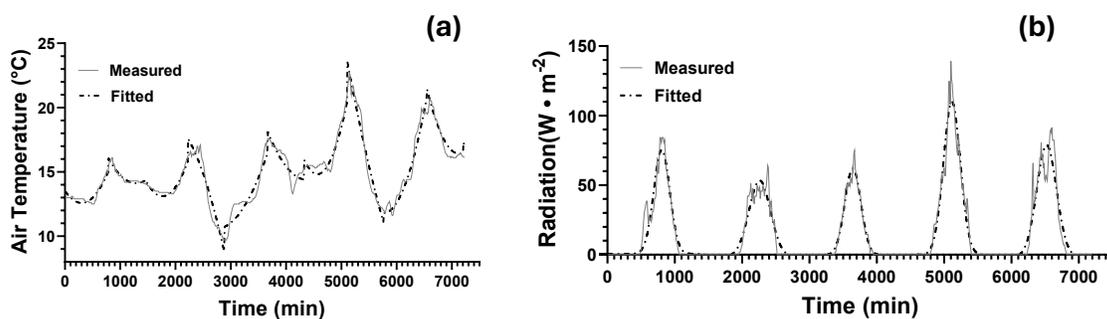


Fig. 22. Evolution of air temperature and radiation inside the greenhouse: measured values (solid line) and model adjustments (dashed line) for (a) radiation during summer and (b) air temperature during autumn.

3.5 Results and discussion.

3.5.1 Water quality parameters

Figure 23 depicts the average, and the standard deviation for water temperature, pH and dissolved oxygen in the different sections analyzed of the hydroponic bed, for the southern hemisphere summer. In the for CWF, no differences were found. The experimental results indicated that the values of the average water temperature during the experiments-ranged from 21.78 to 21.86 °C, while the values of the pH varied between 3.54 and 3.61. Finally, the concentration of dissolved oxygen in the water was between 6.46 and 6.51 mg O₂/L (Figure 23a, c, e).

In the PWF condition, no significant differences were found for the averaged data of water temperature, pH and dissolved oxygen in the different sampled sections of the hydroponic bed. However, the range between the maximum and minimum values was wider. The analysis of the measured results of the averaged values for the CWF condition indicated that the temperature variation was low, with ranges between 20.45 and 20.59 °C, the same occurred for pH that varied from 4.11 up to 4.14, while the dissolved oxygen concentration in the water was in the interval between 4.11 and 4.24 mg O₂/L (Fig. 23 b,d,f). The dissolved oxygen concentration in the water was the variable that experienced the largest difference in the PWF when compared to the results obtained with CWF, due to the slower water flow. These differences between CWF and PWF, for the same season, were due to the higher kinetics of the water mass in the bed for the continuous flow condition. During the autumn season, all water quality variables were uniform, and without significant differences for CWF and PWF. Average water temperature values for CWF ranged from 16.79 to 17.05

°C, and for PWF from 15.67 to 15.75 °C. The pH for CWF varied from 6.52 to 6.57, and for PWF it remained constant in all three zones at a value of 6.56. Finally, the concentration of dissolved oxygen in the water for both flow types varied in the interval between 5.58-and 5.84 (Fig. 23e,f).

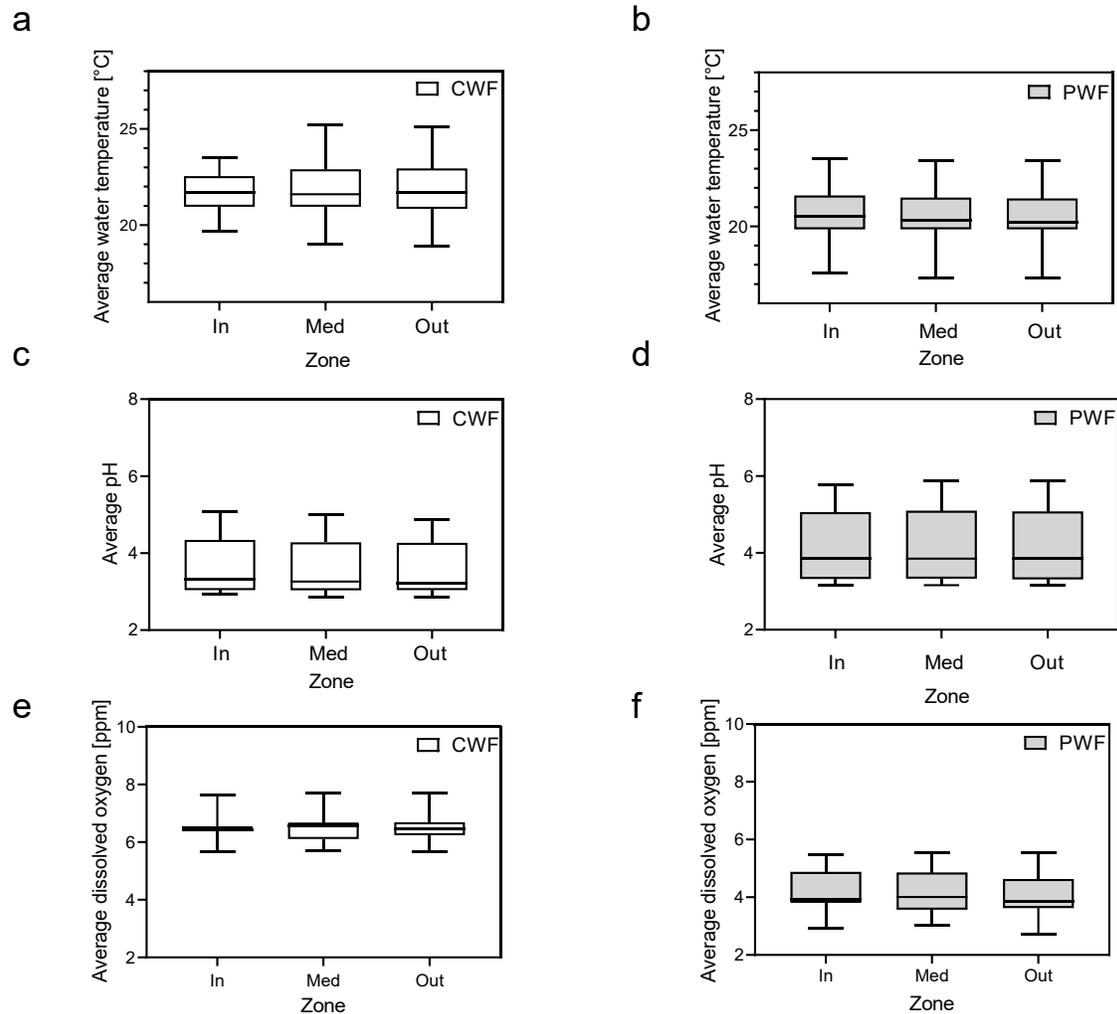


Fig. 23. Average water temperature for (a) CWF, (b) PWF. Average pH for (c) CWF, (d) PWF. Average dissolved oxygen for (e) CWF, (f) PWF in the southern hemisphere summer.

The highest values were found in the zone near the inlet, and the lowest in the outlet. A similar pattern for both types of inlet flows was noticed in summer, that exhibited higher values of temperature than at the hydroponic bed outlet. As expected, average water temperature of the water was higher in summer than in autumn, especially for the CWF water inlet condition. This trend could be detrimental if the value of temperature is higher than the comfort value for this type of lettuce. The variation during the day and night for sunlight, temperature, and humidity, in both seasons, is detailed below (Table 9).

Table 9. Air temperature, solar radiation and relative humidity variation inside the greenhouse, for summer and autumn, during the day-night cycle, where Daytime: 6:00 am to 18:00 pm. Nighttime: 19:00 to 5:59 am.

Weather season	Day - night cycle	Temperature [°C]	Variation	
			Solar Radiation [W/m ²]	Relativity humidity [%]
Summer	Daytime	12.6 to 28	2 to 341	48 to 83
	Nighttime	11.7 to 24.3	2 to 168	57 to 83
Autumn	Daytime	8.4 to 25.7	0 to 178	50 to 80
	Nighttime	8.4 to 18.4	0 to 3.7	60 to 80

Next section analyses the effect of water temperature on lettuce growth.

Detailed water temperature evolution over time for CWF and PWF conditions in summer and autumn is presented below (Fig. 24), highlighting the impact of pulsatile flows (PWF and TWF) in reducing water temperature to levels lower than for continuous flow (CWF). Experimental results showed that the water temperature was

1.5°C higher for CWF compared to PWF during summer. In autumn, a similar trend was observed for CWF, PWF, and TWF, with CWF (Fig. 25), continuous flow, exhibiting the highest average temperature. When comparing seasonal differences, the average water temperature was 5°C higher in summer than in autumn.

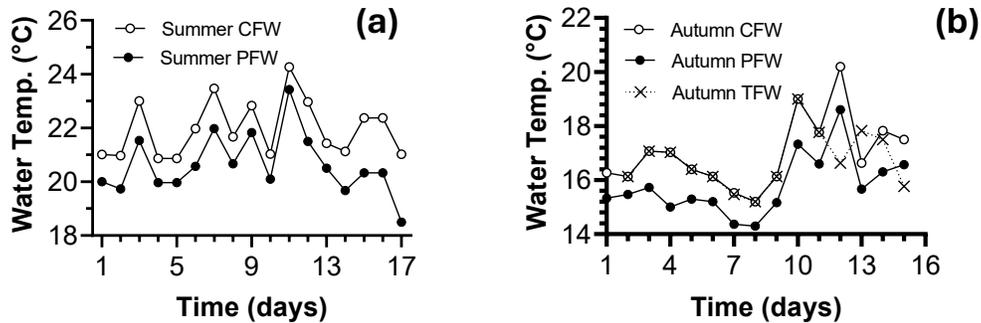


Fig. 24. Evolution of water temperature in: (a) summer for CFW and PFW, and (b) in autumn for CFW; PFW and TWF.

In general terms this temperature increment was caused by the continuous water flow, with a mass flow rate of 0.02 L/s during 24 hours of each day of the growing period. In the pulsed mode (PWF), the water flow, for 20 minutes, was supplied six times a day. Therefore, in this case, water was in steady state during 22 hours per day.

3.5.2 Leaf growth performance

In summer, Figure 25 displays the average leaf growth at the end of farming for CWF was 93.06 ± 21.15 mm, and for PWF it was 112.14 ± 12.05 mm. For both types of inflow, less growth was observed at the central row along the hydroponic bed, being this difference smaller for the PWF condition.

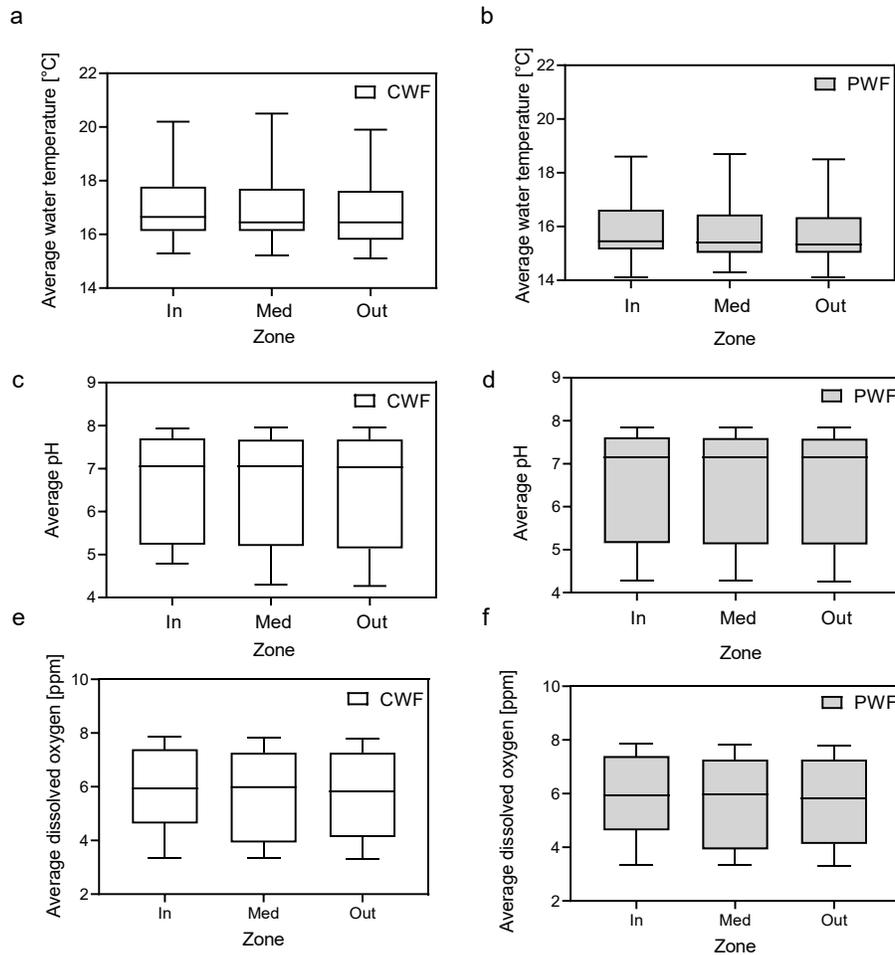


Fig. 25. Average values of temperature water for: (a) CWF and (b) PWF; average pH for (c) CWF, (d) PWF, and average dissolved oxygen for (e) CWF, (f) PWF in southern hemisphere autumn.

In the PWF condition, where the average water temperature was lower than in CWF, the average leaf height at harvest was 17% larger (Fig. 26). This was due, in the PWF, to the closer proximity to the optimum water temperature reported for lettuce in hydroponics (Thakulla et al., 2021).

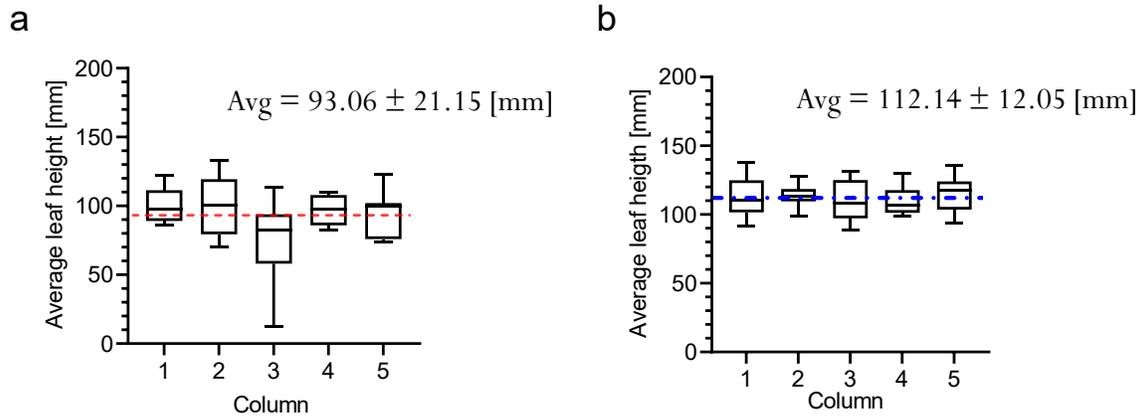


Fig. 26. Leaf growth performance in southern hemisphere summer. (a) CWF. (b) PWF. The dotted line represents the average.

Another factor that contributed to increase the yield and to achieve a more homogeneous growth across the width and length of the bed in PWF was that the lettuces were in contact with the nutrients for a longer time. This was caused by the water flow and explained by the streamlines calculated with the computational model. Experiences related to the time of exposure of the plant to the nutrient were accomplished by Razzaq Al-Tawaha et al., (2018). They reported that water movement in the system and the rate of turnover are crucial factors that need to be carefully designed to ensure an optimal contact time for the interaction between roots and nutrients in the water flow in the hydroponic bed. This is an essential issue for the overall health and productivity of the plants that was predicted by the numerical fluid mechanics model for the water flow.

The analysis of hydroponic lettuce growth in autumn exhibited the same trend than in summer, however the average value of the leaf sizes for both flow types were slightly larger. Figure 27 indicates that for the CWF condition the value of the growth was 115.23 ± 26.30 mm, and for PWF 114.97 ± 12.00 mm. In these two types of flow

the same growing performance as in summer was observed, in which the lettuces located along the central row achieved a larger size, while a lower average growth was observed for the lettuce rows near the hydroponic bed walls. A similar leaf growth performance was observed for CWF and PWF.

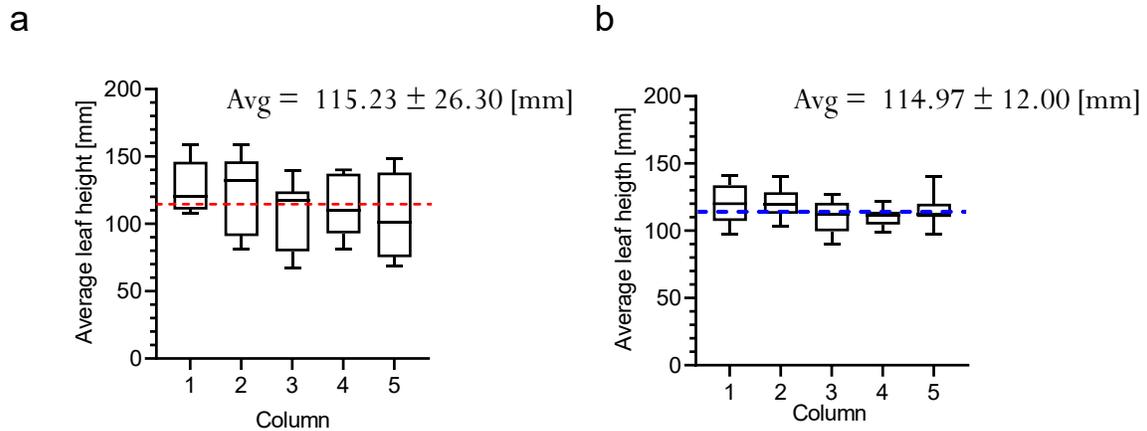


Fig. 27. Leaf growth performance in southern hemisphere autumn. (a) CWF. (b) PWF. The dotted line represents the average value.

Finally, the effect of including two additional inflows, with a total of three inflows (TWF), operating in a pulsatile pattern, resulted in a significant increment of the average leaf size that reached a value of 175 ± 18.45 mm, with an increment of 52% with respect to CWF and PWF.

The impact of the climate season and temperature, on hydroponic crops has been reported in the zucchini squash. The information available indicates that plants grown in the summer-fall season exhibited lower yield and growth compared to those grown in the spring-summer season (Rouphael and Colla, 2005). High temperatures during this season can lead to a more rapid increase in the water electrical

conductivity (EC) of the growing medium when using sub-irrigation compared to drip-irrigation systems. This is due to the fact that the high temperature generated in closed greenhouses, without ventilation, promotes the evapotranspiration of water, generating an accumulation of salts in the nutritive solution (Carmassi et al., 2003).

Specifically in lettuce grown inside greenhouses, the seasonal impact in desert environments was evaluated by Glenn (1984), observing that high temperatures during the summer rainy season were associated with bolting, which negatively affected lettuce growth. Also, daytime air temperatures positively influenced lettuce growth, and there was a significant temperature - radiation interaction. On the other hand, autumn crops grown under higher temperatures, responded more steeply to radiation than spring crops, affecting the harvesting times, that decreased from 62 days in summer to 42 days in autumn.

To visualize the growth differences, for the different water pulse conditions, an average normalized leaf growth, in summer and autumn, was employed using a heat map for the different flow conditions (Fig. 28).

Under CWF during the summer, the heat map shows significant variability in growth (Fig. 29), with alternating areas of high and low yield. This could suggest that, despite the consistency in water flow, the nutrient distribution was not uniform in the bed, and that the higher value of water temperature had a leading unfavorably effect in lettuce growth. In autumn (Fig. 28b), the growth patterns were more uniform, possibly due to the reduced water temperature variation that favored a more homogeneous distribution of the nutrients to the plant roots. Water pulsed flow

system show a clear heterogeneity in growth, indicating that the nutrients may not be efficiently reaching all areas of the hydroponic bed (Fig. 28b).

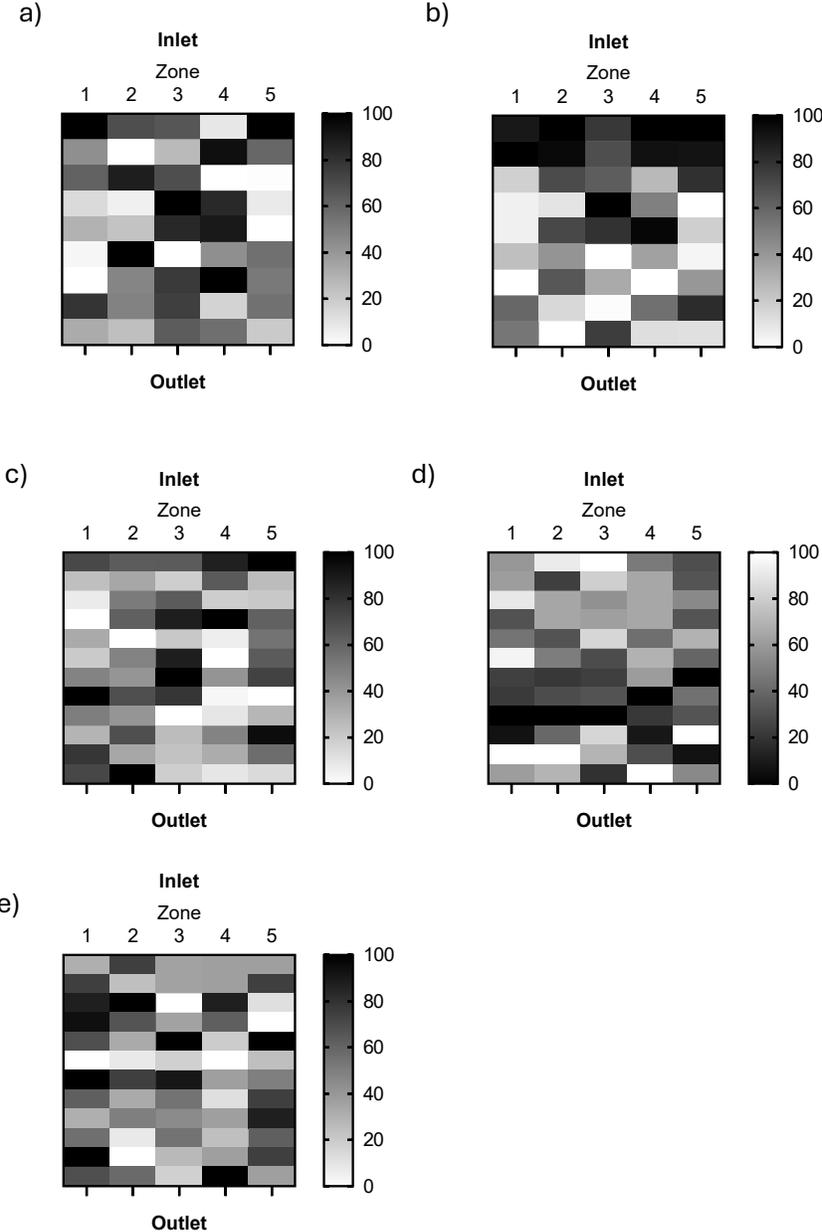


Fig. 28. Lettuce growth, by a heat map graph, for the three-inlet water flow in summer and autumn. a) Summer in CWF, b) Summer in PWF, c) Autumn in CWF, d) Autumn in PWF and e) Autumn in TWF.

This observation highlights the importance of adjusting the frequency and magnitude of pulses to optimize leaf coverage (FGCC). During autumn, the variability in growth decreased, suggesting that cooler conditions improved nutrient dissolution and plant growing, because water and greenhouse temperatures were in the range of thermal comfort (Fig. 28 c, d). The lettuce growth heat map with TWF, which uses a pulsed flow with three inlet points, shows a marked improvement in lettuce length (Fig. 28e), with a more uniform distribution of size compared to the traditional PWF. Although, at the center of the bed there is still a region with a lower growth that is narrower than the observed for the other two flow inlet systems. The use of three inlet points appears to be mitigating the shortcomings of the PWF system, providing an improved pattern growth, which can be originated by the enhanced water circulation in the bed.

3.5.3 Leaf weekly growth

During the four weeks of summer, lettuce growing in two water flow modes: continuous flow (CWF) and pulsed flow (PWF), was compared. In the first week, the bed with pulsatile flow lettuces exhibited greater leaf growth than continuous flow, reaching approximately 45 mm, while the lettuces in the continuous flow recorded a growth of about 35 mm. In the second week, CWF slightly outperformed pulsatile flow, with growth of 25 mm versus 20 mm. In the third week, growth in the pulsatile bed, reached 30 mm compared to 20 mm in the CWF bed (Fig. 29a). Finally, in the

fourth week, PWF continued to show a better performance, with a growth of approximately 15 mm, while CWF remained at 10 mm.

In the autumn, a third flow mode was added to the analysis, TWF. During the first week, both PWF and TWF showed similar growth of approximately 35 mm, outperforming the continuous flow which had a growth of 25 mm. In the second week, TWF showed a remarkable increase in growth, reaching 50 mm, while the PWF recorded 30 mm and the CWF 15 mm. In the third week (Fig. 29b), TWF continued to stand out with 40 mm of growth, followed by PWF with 25 mm and CWF with 20 mm. In the fourth week, TWF showed exceptional growth of 70 mm, well above the 30 mm of the pulsatile and 25 mm of the CWF.

During the summer, PWF consistently favored greater leaf growth compared to CWF. In the fall, the addition of three pulsed water inlets (TWF) had a significant impact on growth, especially in the fourth week. TWF proved to be the most effective in the fall,

During summer, environmental conditions inside greenhouse, particularly temperature, experienced significant variations with peaks reaching up to 30°C. This increment in temperature directly impacted oxygen dissolution and water pH. As observed in the experiments, the concentration of dissolved oxygen decreased drastically, especially in the PWF, where the intermittent flow accentuated this drop, reducing the oxygen availability for the lettuce roots. Additionally, the pH also

decreased, diminishing the nutrient absorption. suggesting that both seasonality and flow type play a crucial role in plant growth (Fig. 29b).

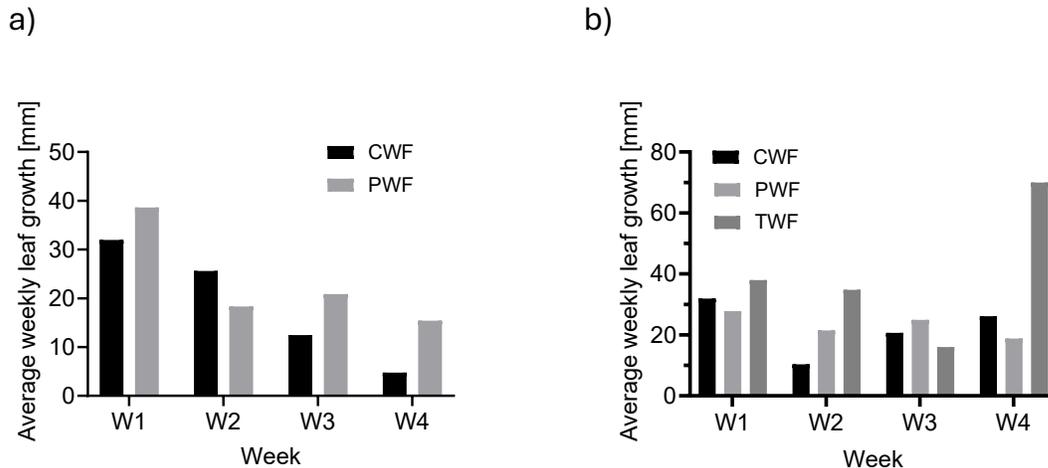


Fig. 29. Weekly lettuce growth rates in summer (a) and autumn (b), for the continuous (CWF) and pulsed flow variants (PWF - TWF).

Under these conditions, it was understandable that leaf growth was more affected in PWF than in CWF, given that CWF provided a continuous water flow that could partially mitigated the adverse effects of low oxygenation and pH fluctuations. In contrast, during the autumn, the temperature was more stable and significantly lower, with values ranging between 15°C and 20°C. These milder conditions allowed both, CWF and PWF, to maintain higher values and more constant levels of dissolved oxygen in the water, while the pH level did not experience significant fluctuations. As a result, both flow systems provided similar conditions for nutrient and oxygen absorption by the roots, which was reflected in a similar leaf growth between the two systems during this season.

3.5.4 Leaf/Root ratio performance

A dimensionless indicator was used to evaluate the lettuce growth performance of the leaf versus the root (L/R ratio). This indicator was calculated by dividing the height of the leaf by the height of the root. The dimensionless indicator Leaf/Root ratio value varied in the interval between 0.7 to 1, where lower numbers indicated a larger size of the root compared to the leaf.

The trend of this indicator for CWF is that in the columns near the edges, columns 1 and 5, the average leaf height was significantly higher than the average root height in summer and autumn. This finding could be related to water dynamics and the effect generated in these zones by the water moving along the bed. This flow pattern will be evaluated by CFD fluid mechanics in section 3.4.

In summer, this indicator shown in Figure 30 was the same for CWF and PWF, however the variation of this indicator along the five rows was lower in the PWF hydroponic bed.

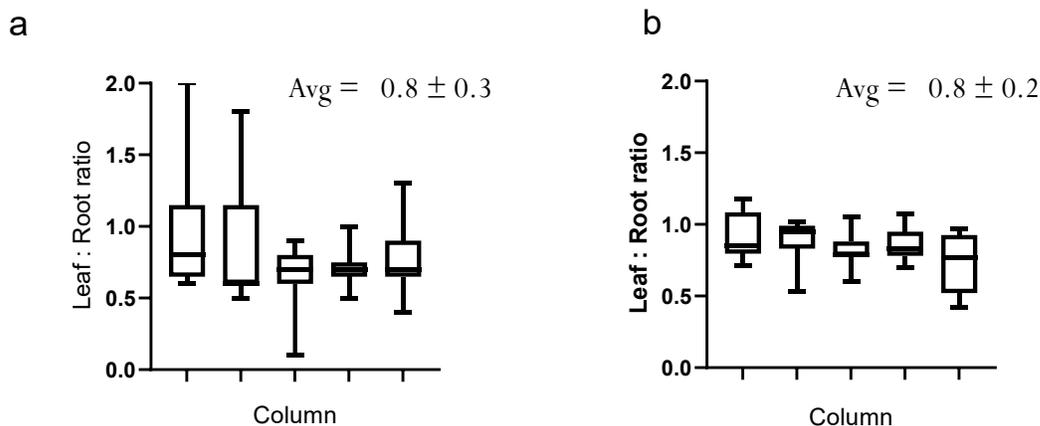


Fig. 30. Leaf/Root ratio performance in southern hemisphere summer. (a) For CWF. (b) For PWF. The dotted line represents the average.

On the other hand, as illustrated in Figure 31, in autumn the L/R ratio was the same for both types of flows, CWF and PWF. In the TWF configuration the L/R ratio was the lowest, i.e. here the roots showed a greater development than in all the other configurations.

When comparing roots from hydroponically grown and soil-grown lettuce Lei & Engeseth (2021) found that hydroponically grown lettuce had longer roots, higher moisture, and lower ash compared to soil-grown lettuce. Also, they determined that hydroponically grown lettuce had softer leaves but firmer leaf midribs compared to soil-grown lettuce, possibly due to the higher lignin content in hydroponically grown lettuce (Lei and Engeseth, 2021).

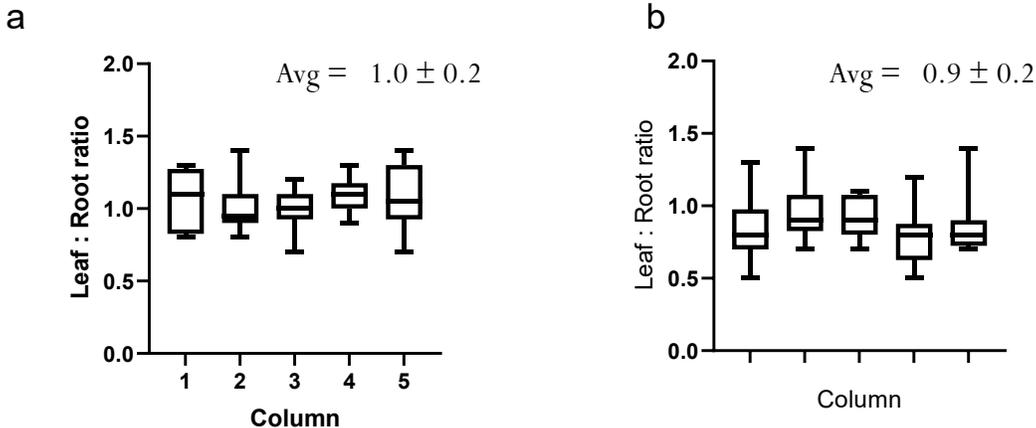


Fig. 31. Leaf/Root ratio performance in southern hemisphere autumn. (a) For CWF. (b) For PWF. The dotted line represents the average.

The findings in—our research based on the assessment of the different flow conditions, such as CWF, with one water inlet, versus TWF, with three water inlets,

generated different physiological responses in roots and leaves being provoked by the water flow that transported the nutrients along the streamlines and its interaction with the growing lettuce roots. Baiyin et al., (2021) reported the visualization of the hydroponic nutrient solution and flow patterns by particle image velocimetry to assess an ideal water flow and nutrient trajectory resulting in an improved plant growth with elongated roots. Also, they indicated that increasing the flow rate promoted the plant growth, for flow ranges between 4 to 6 L/min where the ideal flow rate originated an improved nutrient absorption and plant growth. However, it is important to notice that the ideal flow rates for roots in hydroponic systems should vary depending on the plant species and growth stage.

Some interesting results have been provided for a recirculating aquaponic system by Nuwansi et al., (2016), in which they evaluated the effect of water flow rates in a recirculating aquaponic system that produced fish and spinach. They found that maintaining a flow rate of 0.8 L/min resulted in the highest values of plant height, percentage height gain, and yield of spinach. Additionally, as the water flow rate decreased, there was an increment in plant growth and nutrient uptake, specifically nitrogen, phosphorus, and potassium. Also, the water retention in the tanks increased, allowing for longer periods of nutrient uptake. This is particularly beneficial in low flow rate systems. The extended time in the tanks ensures that plants have ample opportunity to absorb the necessary nutrients for a healthy growth.

On the other hand, Endut et al., (2011) in an aquaponic recirculating system of water spinach reported that high flow rates, between 1.6 to 2.4 L/min results in an improved nutrient removal and a potentially enhanced plant growth.

In the NFT technique, which is developed using pipelines, empirical observations revealed that lettuce growth decreased with increasing water flow rate (over 0.5 L/min), and enhanced the lettuce growth when the water flow rate was 20 L/h (0.3 L/min)(Razzaq Al-Tawaha et al., 2018).

The water flow rate results of the last four cases discussed do not apply directly in DWC hydroponic culture. However, these results provide a valuable basis for understanding the effect of water flow on nutrient transport by the DWC technique, and how these nutrients affect root and leaf growth.

3.5.5 Fluid mechanics in water CWF hydroponic tanks

The fluid mechanics results of the water flow in the hydroponic beds obtained from the computational model in the CWF hydroponic bed flow are analyzed and discussed here. Figure 32 shows high velocity concentrations in the area close to the inflow that decreased slightly as the flow moves along the rear side of the hydroponic bed. The streamlines indicate drag patterns that would allow the removal and movement of nutrients, being concentrated at the effluent section.

The range of velocities calculated with the computational model at mid-water height, at 5 cm depth, was between 0.02 and 0.06 cm·s⁻¹ in the first third of the bed near the affluent. At the mid-section of the hydroponic bed, the values were in the range of

0.01 to 0.02 cm·s⁻¹, towards the effluent section. This drag pattern inhibits the nutrients to remain in the hydroponic bed for some time, reducing the growth of the lettuce, especially at the central area, along the length of the hydroponic bed. The presence of dead zones, with negligible water flow, resulting from the non-generation of recirculation patterns, was predicted by the fluid mechanic model. This action provoked smaller crop sizes in specific areas of the hydroponic lettuce bed, as depicted in Figure 32.

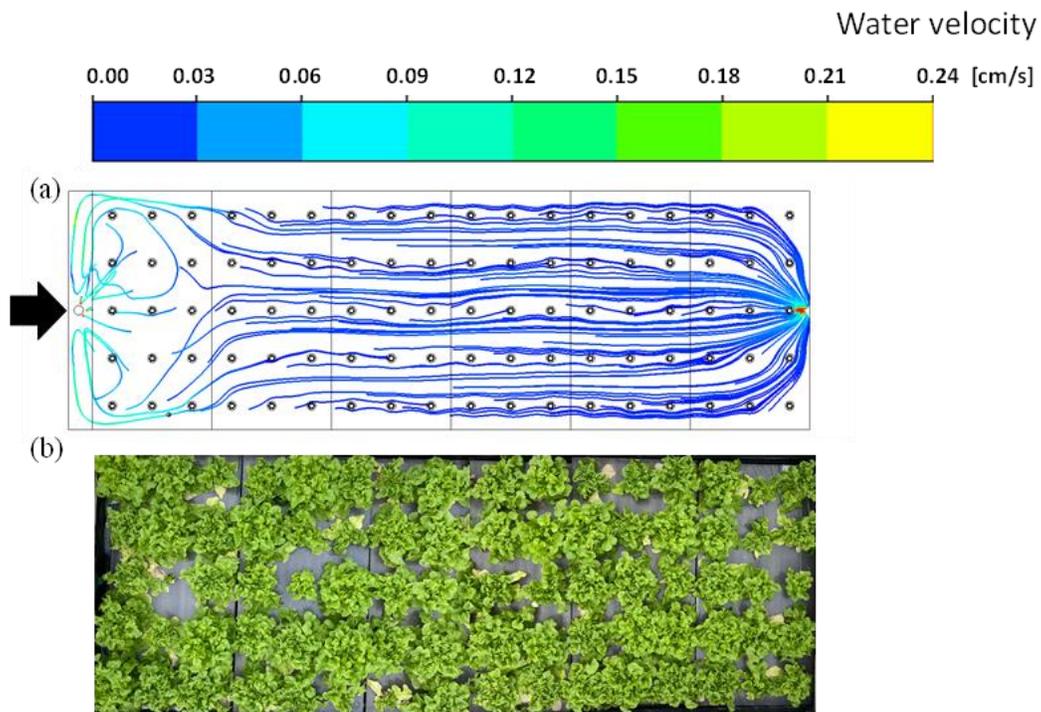


Fig. 32. (a) Results of the fluid mechanics model and (b) growth of lettuce in CWF, obtained in southern hemisphere summer.

The analysis of the different water flow patterns and streamlines, allowed to estimate where the nutrients that are transported by the water movement would remain for a

longer or shorter time depending on the flow strategy, the water recirculation, as well as the described streamlines that directly influenced the lettuce roots growth.

Use of a constant flow water (CWF) and higher velocities in our experiments originated a negative impact on nutrient transport, and on lettuce growth, for leaf and root. This result is in agreement with the findings of (Razzaq Al-Tawaha et al., 2018) using NFT technique in lettuce. They found that water flow in the NFT with a rate of 20 L/h was found to improve lettuce hydroponics growth over either 10 L/h or 30 L/h, in terms of the number of leaves and height.

The number and size of the roots of lettuce, grown by hydroponics, play an important role in the coloration of the plant, originating differences that were noticed in our study. Suo et al., (2021) indicated that retaining the primary or whole root of hydroponic lettuce helps to reduce the leaf color difference, and that the optimal length of root retention for hydroponic lettuce is 9 cm.

Determining the type of water flow in terms of the spatial three-dimensional distribution of the three velocity components, streamlines and recirculation zones in the hydroponic bed as well as designing a suitable water inflow strategy was achieved by the CFD models and computational simulations and verified by physical experiments.

3.5.6 Fluid mechanics of water in hydroponic bed with PWF flow

The computational modelling of water flow in lettuce hydroponic bed, depicted in Fig 17, allowed to identify the generation of water recirculation zones at the beginning

of the hydroponic bed and at the middle zone, specially for the PWF inlet flow condition. The streamlines described the recirculating movement of the nutrients being transported in the water.

The average velocity was calculated by the results of the CFD simulations, as the mean value of the horizontal velocity component for a plane at mid – height of the water in the hydroponic bed. The values of streamlines predicted by the fluid mechanics model, and the experimental results of foliar green cover (FGCC) illustrated in Figures 16, 17 and 18, for CWF, PWF and TWF, respectively, corresponds to the data of May 15th, 2023, at 12:00 hours (midday), 17 days after transplantation

The range of velocities predicted in these sites, at mid-water column height, was in the range of 0.005 to 0.01 $\text{cm}\cdot\text{s}^{-1}$. In the areas where the vortices were not observed, the velocity was close to zero. Comparing the average water velocity along the entire hydroponic bed between the CWF and PWF flow conditions, a decrease of approximately 50% was observed for the averaged value of water velocity in the PWF system. This finding is in accordance with what was noticed in the plants for this condition, where a more homogeneous growth was observed for the PWF than in the bed with CWF inlet water flow. However, a slightly lower coverage was still located at the central zone of the PWF hydroponic bed. The water flow patterns, represented by the streamlines, that are tangents at each point of the water velocity vector, predicted by the computational model, indicated the presence of dead zones. These zones were coincident with the presence of a significantly lower lettuce growth in these same zones. On the other hand, the evident recirculation calculated by the

model coincided with the zones of larger growth in the crop, as it can be noticed in Figure 33.

Previous studies have shown that the decrement of the water flow rate can enhance plant growth and nutrient uptake, while increasing the flow rate can have negative effects on plant growth. (Vega et al., 2023) found that intermittent circulation had higher yield per energy input for pepper culture in DWC. Additionally, a constant circulation of nutrient solution was found not being beneficial.

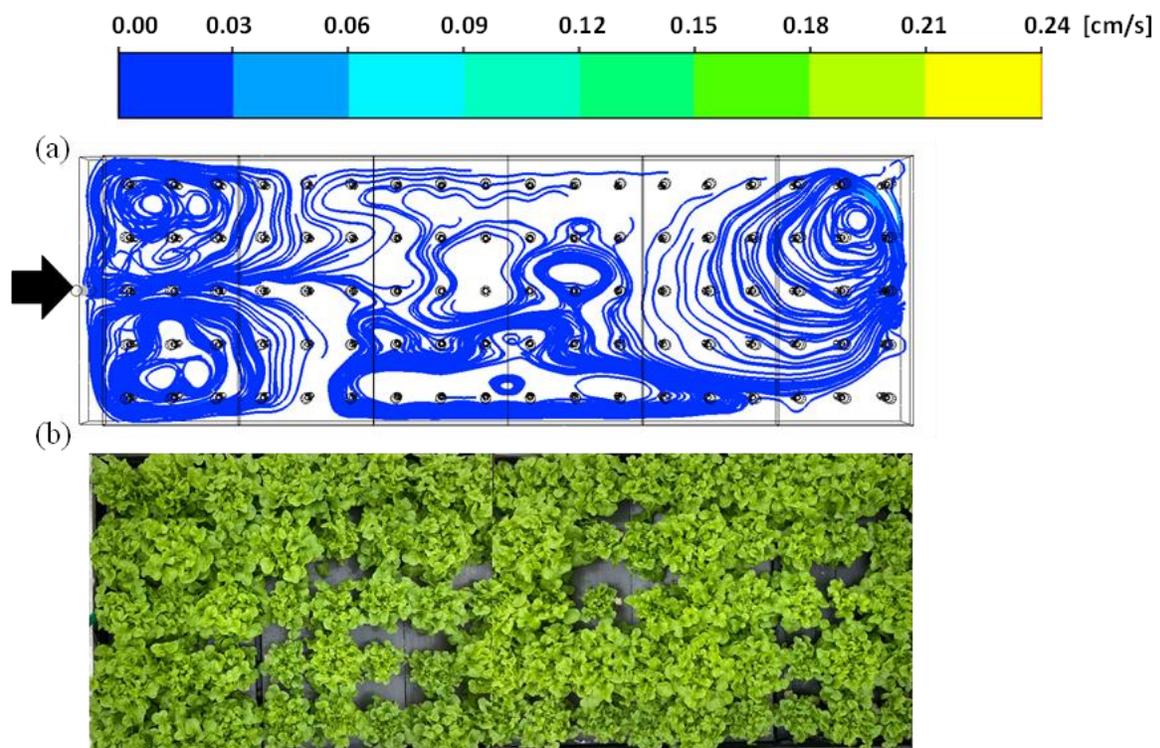


Fig. 33. (a) Streamlines predicted by the fluid mechanics model, and (b) growth of lettuce in PWF, obtained in southern hemisphere summer.

3.5.7 Fluid mechanics in hydroponic bed with TWF water flow

Finally, for the TWF condition, streamlines with velocities similar to those calculated for the CWF can be observed in Figure 34. As a result of the two additional water inlets, a braking effect on the water flow was produced, and hence that the trajectory and velocity of the influent flow was beneficially affected. The lettuce could be exposed to the transport of nutrients in a stable and homogeneous way, which resulted in a more homogeneous leaf cover than in the two previous inflow cases analyzed. The velocity ranges for the streamlines generated at mid-water height that are clearly identified in three zones of the hydroponic bed by their order of magnitude. At different depths, no different velocity profiles were found in the fluid dynamic model, attributable to the shallow depth, 10 cm of the hydroponic bed, and to the low water velocity. The highest values of velocities calculated were mainly concentrated at the sector near the last board and close to the hydroponic bed effluent, with ranges that were between 0.02 and 0.04 $\text{cm}\cdot\text{s}^{-1}$. In the central zone, close to the two lateral affluents, the velocity values were between 0.01 and 0.02 $\text{cm}\cdot\text{s}^{-1}$. Finally, at the zone of the head affluent the velocity values were extremely low, and varied between 0.007 and 0.01 $\text{cm}\cdot\text{s}^{-1}$. The flow pattern represented by the streamlines in the model demonstrates that a more homogeneous flow originated appropriated values of water velocity and circulation patterns that allowed an efficient transport of nutrient towards the crop roots, that contributed to an increased lettuce growth in the hydroponic bed, as depicted in Figure 18.

The proposed experimental and modelling hybrid methodology, based on mathematical simulation of fluid mechanics and heat transfer phenomena in

hydroponic crops, allowed to predict the water velocity distribution, streamlines, recirculation zones and temperature gradients. Hence, these findings were contributing to improvements in the decision-making process in relation to greenhouse thermal management, hydraulic configurations and implementation of cultivation strategies in these systems.

3.5.8 Heat transfer in CWF, PWF and TWF

The analysis of the convective heat transfer phenomena in the CWF, PWF and TWF hydroponic beds by means of computer modelling was contrasted in Figure 34 with the average temperature measured in the water between 27 April to 30 May. At the beginning of the experiment, the starting temperature was 15.2°C. The calculation of the heat transfer results was carried out from 12 May to 17 May of 2023, due to the significant experimental variation of water temperature observed in these days.

In the six days in which the evolution of water temperature was evaluated with the unsteady heat transfer model, the average value for PWF was 17.02°C, while for CWF was 1°C higher (17.98°C). The lower temperature of water inside the hydroponic bed originated by the pulsatile three inlets flow contributed to improve lettuce yield. Average water temperature, between the 12 and 17 of May, of CWF reduced from 17.7 °C to 16.7 °C for PWF, and to 15.9 °C for TWF, during the autumn season. The highest lettuce yield and FGCC, and the fastest growing rate were

obtained with the lower average water temperature, by using the three-inlet water flow mode (TWF).

Figure 34 indicated that the overall trend of the average water temperature from 12 and 17 of May 2023, predicted by the mathematical model is in qualitative and quantitative agreement with the experimental results, for the CWF (Fig. 34a), PWF (Fig. 34b) and TWF (Fig. 34c). The water temperature pattern responds to the average daily fluctuations recorded at the station, outside the greenhouse. The improved water flow pattern of the three inlets pulsatile flow over the other two alternative flows with one inlet (CWF and PWF), originated an enhanced nutrient distribution inside the hydroponic bed. Therefore, the prediction of fluid mechanics and heat transfer by mathematical modelling and computational simulation for water and energy flows and inside the hydroponic beds, predicted the evolution of water velocity and temperature distributions inside the hydroponic bed, contributing to explain the experimental findings of the higher yield, leaf and root size of lettuce.

The solution of the mathematical model of the forced convective heat transfer in the water by the finite volume method allowed to incorporate the effects of environmental conditions inside the greenhouse in the prediction of the evolution of temperature distribution of water inside the hydroponic bed. The experimental findings indicated that there is an inverse relationship between lettuce leaf and root growth and water temperature.

Average water temperature in the five days growing time (12 and 17 of May 2023) was 0.6 °C lower than the average temperature of the wood sides of the hydroponic

bed. Thermal capacity of water in the bed originated a delay that changed from 2 to 4 hours respect to the variation of the air temperature inside the greenhouse.

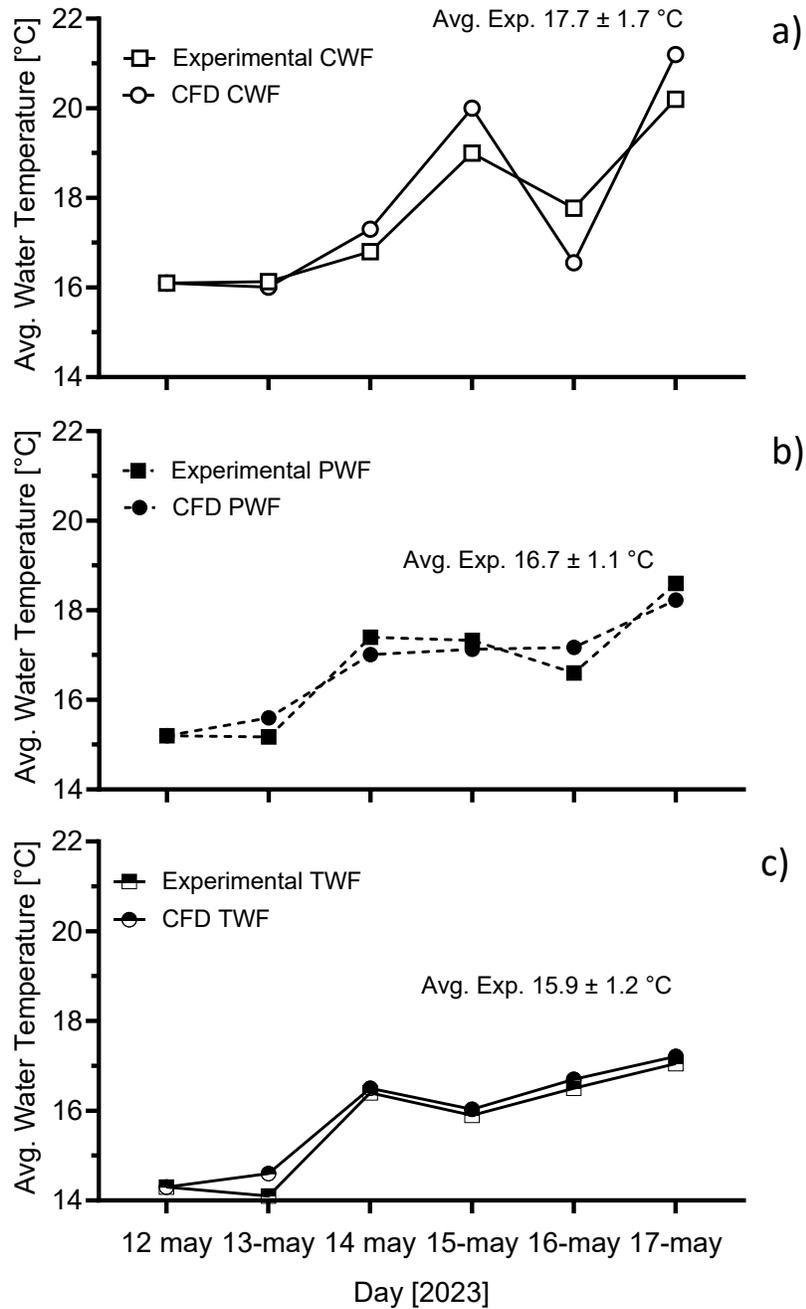


Fig. 34. Average predicted and experimental water temperature in autumn for a) CWF, b) PWF, c) TWF.

3.6 Statistical analysis

3.6.1 Growth and Water quality

A repeated measures Analysis of Variance (ANOVA) and a Multivariate Analysis of Variance (MANOVA) with multiple comparisons with Bonferroni adjustment were used to evaluate the evolution of water quality on lettuce growing. The length of leaves was evaluated for half of the crops in each hydroponic bed. Both statistical analyses were applied to evaluate the effect of the distribution of temperature, dissolved oxygen concentration, pH and electrical conductivity in the water on the evolution of growth at the three beds zones (inlet, middle, end) in relation to the continuous water flow used as the control.

3.6.2 Growth summary

ANOVA and MANOVA analysis, at summer and autumn, evaluated for all water inlet conditions (CWF, PWF and TWF), revealed significant effects of time on the evolution of lettuce growth ($p = .000$), as it is described in the next section.

3.6.3 Growth in summer and autumn

In summer, for CWF, the unique statistically significant difference was found between zones 1 (inlet) and 3 (outlet) with a p-value of .022, by the results of Bonferroni adjusted multiple comparisons. However, for PWF no significant interaction between time and bed zones were observed ($p > .146$), indicating that the temporal variation was uniform across different bed zones for this type of water flow.

On the other hand, in autumn, interactions between growing time and hydroponic bed zones were not detected. Statistical results indicated p-values greater than 0.146 in all tests for CWF, higher than 0.385 for PWF and larger than 0.182 for TWF. The trend of the results shows that the effect of time is uniform across all the beds for the three water flows studied. Bonferroni adjustment confirmed the absence of statistically significant differences between of growing between all zones for each CWF, PWF and TWF in the autumn, with all p-values being equal to 1.000.

3.6.4 Water Quality in summer and autumn

Water temperature, dissolved oxygen concentration, pH and electrical conductivity (EC) experienced significant differences during the growing period ($p=0$). This analysis evaluated differences at the three zones of the hydroponic bed (In, Med and Out), with the major differences found between the inlet and outlet zones.

During summer the evolution of the four water quality variables in the bed with CWF registered significant differences at the three bed zones over time ($p < .001$). In contrast, for PWF the only variables that changed with time in the bed zones were water temperature ($p = 0.017$) and EC ($p=0.009$).

On the other hand, in autumn, using Roy's Largest Root criterion, it was found that for CWF there are differences between zones, with a moderate effect on the measured parameters ($p = .006$). For PWF, no significant differences by zones were observed for pH and dissolved oxygen concentration (DO). However, significant

temperature differences were noticed between the inlet and outlet zones ($p = 0.007$), as for the EC ($p = 0.008$). Lastly, for TWF, no significant differences were found for any of the variables studied over time across the three zones (DO $p = 0.551$, the p -values obtained for the three relevant parameters were: water temperature $p = 0.576$, pH $p = 0.527$, and EC $p = 0.234$).

3.7 Preliminary conclusions

This study evaluates hydroponic growth of lettuces in southern hemisphere during summer and fall inside a greenhouse by a hybrid experimental-computational modeling approach to determine the effect of water inlet flow and temperature on the cultivation time and yield. A pulsating flow (PWF), with three water inlets (TWF), in autumn, originated larger lettuces, in a shorter growing period, with more homogeneous leaves compared to the continuous and pulsatile flows with only one inlet. The results obtained experimentally indicated that both: root and leaf size of hydroponic lettuces, reached a 25% increment in length by the pulsed waterflow condition with three flow inputs (TWF). This increased length was achieved in both climatic seasons evaluated. In addition, for this same inlet flow condition the plant achieved a larger fraction green cover canopy (FGCC), indicating that its growth was proportional in all three dimensions. The possibility of the development of favorable variations in water quality parameters and nutrients transport to the crop roots in the vertical and horizontal directions is also evident. These variations can be mitigated

or accentuated by the water in let strategy, where the three-inlet variant proved to be the most favorable for the distribution of water quality parameters.

The CFD prediction of fluid mechanics allowed to describe the water flow inside the hydroponic beds and hence helped to understand the nutrients transport to the lettuce roots. This development opens the possibility of using data obtained by automatic measurement as a power input for computational models.

Limitations of findings included a lack of quality issues related to the lettuce properties and water supply; the absence of biology and chemical knowledge in the mathematical model, and further experiments to relate the lettuce growth to temperature, oxygen, pH, water velocity and nutrients flows in water, roots and leaves, combined with plant respiration and photosynthesis. Future studies should incorporate the measurement of organoleptic and nutritional properties of the crop and a detailed information of traces of pollutants in water (heavy metals and arsenic), and emergent contaminants such as those of pharmaceutical origin. The ways proposed to overcome the limitations and drawbacks of the study include refining the computational model to account for dynamic changes in biological factors like nutrient uptake and oxygen consumption, that would further enhance the model accuracy and relevance. Additionally, conducting the study year-round across all four seasons would provide valuable insights into seasonal variations and in hydroponic production

CHAPTER 4

TROUT AND LETTUCE GROWING PERFORMANCE IN RECIRCULATION AQUAPONIC SYSTEM

4.1 Abstract

This study evaluates the effects of nutrient dynamics and water quality on the growth performance of lettuce (*Lactuca sativa*) and rainbow trout (*Oncorhynchus mykiss*) in two coupled Recirculating Aquaponic Systems (RAS), both sharing the same design but operating under different thermal conditions and cultivation scales. RAS-A, under controlled temperature (18 ± 2 °C), had twice volume of RAS-B, which was exposed to natural ambient conditions (15 ± 9 °C). RAS-A comprised four pilot-scale subsystems, while RAS-B included two. Within RAS-A, one subsystem (S0) operated without plants, serving as a control to examine nutrient dynamics in the absence of plant uptake. The remaining subsystems (S1, S2, and S3 in RAS-A and both in RAS-B) incorporated lettuce cultivation. Water quality parameters, including pH, Temperature, Electrical Conductivity, Alkalinity, Nitrate, Calcium, and Potassium, were monitored over eight consecutive months. In RAS-A, subsystems S1 and S2 maintained greater pH stability, which favored better lettuce growth. In contrast, S0 exhibited a significant pH decline (from ~8.0 to 6.3) along with marked accumulation of Calcium (+130%) and Nitrate (>200 mg L⁻¹), which adversely affected root development. Trout growth across all subsystems followed a cubic growth pattern ($R^2 > 0.98$; RMSE < 9), with the S0 subsystem in RAS-A, displaying higher initial biomass (+80%) but lacking plant-mediated nutrient removal. Notably, the growth variability of fish differed between systems: RAS-A offered environmental control, but with larger heterogeneity. Conversely, RAS-B originated homogeneous growth, by uncontrol temperature. This work highlights the importance of plant integration and environmental consistency in water quality and growth performance for aquaponic systems.

4.2 Literature review

Aquaponics emerges as an innovative agricultural practice that integrates aquaculture and hydroponics, offering a sustainable solution to the main pressing global challenges such as food security and environmental degradation (Ibrahim et al., 2023). By combining fish production with hydroponic vegetable cultivation, this system allows the transformation of fish waste into nutrient-rich fertilizer for plants, optimizing the use of resources and embodying the principles of sustainability (Reyes Lastiri et al., 2018). Its adaptability supports economic and social development across diverse regions, particularly in developing countries, where it addresses relevant critical issues such as high fertilizer costs and limited freshwater resources. As a model of sustainable food production, aquaponics holds a significant potential to drive global agricultural development and enhance the resilience in the face of environmental and economic challenges (Cifuentes-Torres et al., 2021; Okomoda et al., 2023).

The core of aquaponics is an integrated closed-loop system, where fish excreta are biologically converted into nutrient-dense fertilizers through microbial nitrification processes, facilitating the plant growth, while the plants, in turn, act as biofilters, removing excess nutrients and maintaining the required water quality for aquaculture sustainability (Gullian Klanian et al., 2018). This synergy maximizes resource efficiency, reducing waste and environmental impact (Asadullah et al., 2020). The improved versatility of this hybrid system allows the implementation in both urban and rural settings, making it particularly advantageous in regions grappling with food shortages or pollution, by the adaptivity to various environmental conditions. Aquaponics can be easily applied in a variety of environmental conditions, supporting local food systems and promoting the ecological balance (Beebe et al., 2020; Pérez-Urrestarazu et al., 2019; Turkmen, 2006).

In aquaponic systems, fish waste provides key nutrients like Nitrogen and Phosphorus, while Potassium is often deficient, limiting plant development and system productivity. This requires interventions to improve potassium availability (Duarte & Cerozi, 2024; Harika, Verma, et al., 2024; Wenzel et al., 2021). To address

the Potassium shortfall, in some cases researchers have explored the use of specially formulated fish feeds that are enriched with Potassium. The application of Potassium-enriched feeds has shown promising results in increasing the plant biomass production, thereby contributing to the efficiency and sustainability of the aquaponic operations (Duarte & Cerozi, 2024; Stathopoulou et al., 2021).

Furthermore, aquaponic systems offer a promising alternative to soil cultivation, promoting an increased Chlorophyll production and the augmented photosynthetic capacity in plants (Tsoumalakou et al., 2022). Recent research indicates that these adaptations lead to achieve higher growth rates and greater edible biomass, positioning aquaponics as a viable method for implementing a sustainable agriculture (Isaev et al., 2023). The ability of plants to grow well in aquaponic environments underscores the potential for higher yields compared to the conventional farming practices.

Efficient nutrient utilization is a key factor to the aquaponic success, as well as to improve the plant selection, addressing the pertinent nutrient limitations to enhance productivity and sustainability (Teng et al., 2024; Zhang et al., 2021). This integrated system recycles nutrients between aquatic and terrestrial organisms, minimizing waste and advancing the sustainable food production, particularly through lettuce cultivation (Pinho et al., 2024).

An adequate aquaponic system design strongly influences nutrient dynamics resulting in an improved lettuce yield. Coupled systems often face nutrient limitations due to a shared nutrient pool, restricting plant growth. In contrast, decoupled systems enable the precise nutrient management and allows to achieve yields comparable to hydroponics, with the additional advantage of offering an improved flexibility to meet the plant needs with a high efficiency (Aslanidou et al., 2023). Additionally, maintaining an appropriate value of the fish-to-plant ratio is critical for the overall system balance; mismatched ratios can lead to nutrient deficiencies or the accumulation of toxic substances, both of which adversely affect the lettuce growth and the overall system performance (Colt & Semmens, 2022; Pineda-Pineda et al., 2017; Velichkova et al., 2019).

Trout farming generates nitrogen and Phosphorus-rich effluents that support plant growth in aquaponics. Plants act as natural biofilters, improving the water quality and promoting trout health, making aquaponics an effective dual-purpose system for aquaculture and agriculture (Buzby et al., 2017). Higher stocking densities have been shown to reduce environmental impacts per unit of fish produced, highlighting the efficiency of the use of resources in such systems (Birolo et al., 2020). Nutrient recycling is a critical aspect of aquaponics, where fish waste provides essential nutrients for the cultivation of lettuce, thereby diminishing the reliance on artificial fertilizers (Effendi et al., 2017). This innovative approach not only enhances plant growth but also promotes sustainable agricultural practices. Moreover, recirculating systems are designed to conserve water, utilizing significantly less than the required in the traditional farming methods, which is increasingly crucial in the current context of global water scarcity (Maucieri et al., 2018; Tetreault, Fogle, & Guerda, 2023).

However, the implementation of aquaponic systems is not without challenges. An efficient design is essential to ensure an harmonious balance between the needs of fish and plants, which requires the meticulous control over hydraulic retention times and the efficiency of the waste treatment processes (Tetreault, Fogle, & Guerdat, 2023; Tetreault, Fogle, Fogarty, et al., 2023).

Aquaponic systems are particularly advantageous in water-scarce regions due to their efficient water usage, consuming substantially less water compared to the traditional aquaculture methods (Vasdravanidis et al., 2022; Yep & Zheng, 2019). Additionally, life cycle assessments (LCA) reveal that aquaponics reduces greenhouse gas emissions, offering a lower carbon footprint relative to conventional farming systems (Bordignon et al., 2022). These environmental benefits position aquaponics as a promising solution for sustainable aquaculture, especially in those that integrate cold-water species such as trout and fast-growing horticultural crops such as lettuce.

A careful literature review of aquaponics systems reveals that significant knowledge gaps persist regarding the biomass production, evolution of water quality and nutrient balance over time. Most studies focus on open or semi-closed systems,

limiting the understanding of how certain compounds adjust and accumulate over prolonged cycles. Novel results generated included the dynamical variation of the relationships between pH, NO₂ – N vs NO₃ - N in the aquaponic trout – lettuce system, during a complete growing cycle of trout, with and without lettuce crop and the comparative assessment of growth performance of lettuce - trout under controlled and natural thermal conditions.

4.3 Goal of the study

The objective of this study is to evaluate the effects of water quality and nutrient availability on the growth of lettuce (*Lactuca sativa*) and rainbow trout (*Oncorhynchus mykiss*) in two coupled Recirculating Aquaponic Systems (RAS). One of the systems was specifically used to monitor the water quality parameters and nutrient concentrations to assess their impact on the biomass production. Both experimental systems operated concurrently at the same time but at distinct physical locations and were used to evaluate growth performance of lettuce and trout; however, they differed in their environmental conditions: one system operated under controlled temperature, at 18°C, while the other was investigated at natural ambient conditions.

4.4 Material and Methods

4.4.1 Experimental design

The experiment involved a comparative evaluation of two Recirculating Aquaponic Systems (RAS) with identical configurations, both located in the semi-arid Coquimbo Region, Chile, 100 km apart. The key difference between the systems was their thermal regime: RAS–A operated under controlled temperature conditions, maintained at 18 ± 2°C using a chiller-type cooling system, located at the coastal zone, by the Pacific Ocean, while the RAS–B system functioned under natural thermal conditions, 15 ± 9°C, being in a rural area.

RAS–A operated at the facilities of the Universidad Católica del Norte, in the city of Coquimbo (29.96°S, 71.34°O), whereas RAS–B was located in the rural area of Pan de Azúcar, approximately 100 km from RAS–A (30.05°S, 71.23°O). Both systems were protected from solar radiation, with the aquaculture units covered with Rachel-type mesh structures. The horticultural units were housed in plastic greenhouses with a volume of 177 m³, built by three-layer of polyethylene film (130 microns thick), with a two-season UV additive and 89% light transparency.

The two systems operated simultaneously between September 2023 and June 2024, covering the spring, summer, and autumn seasons in the southern hemisphere. During this period, environmental variables such as air temperature ($\pm 0.1^\circ\text{C}$), solar radiation ($\pm 1 \text{ W/m}^2$) and relative humidity ($\pm 0.1\%$) were recorded using a meteorological station, both located in the proximity of each system. These stations were equipped with sensor Vaisala/HMP50, and the data was recorded by means of a Campbell CR1000 datalogger.

During the period between November 2023 and June 2024, the weather conditions recorded at the RAS – A and RAS – B meteorological stations showed marked differences associated with the location. At RAS – B, the average temperature ranged from 11.0 °C to 19.5 °C, with an average value of 15.3°C, that exhibited a seasonal pattern that peaked in February, coinciding with the highest radiation values in summer (238.3–236.6 W m⁻² in December and January) and a gradual decrease towards winter. The relative humidity (RH) varied from low values in April (26.2%), and a high level of 64% in March. In contrast, at RAS - A, despite fluctuations in the incident radiation—with higher values in summer close to 245 W m⁻² and lower winter solar irradiation around 80.2 W m⁻². The registered data for temperature was more uniform than at RAS - B, with a smaller temperature difference, in the range between 6.4 and 16.2 °C, and an average value of 11.3 °C, than at RAS - B. The RH at RAS - A exhibited less variability (47.7–61.3%), which was due to its proximity to the ocean coast, unlike what was observed at RAS -B, located in an exposed and rural area. These seasonal and geographical dynamics of temperature and air humidity shaped the environmental conditions under which

the aquaponic systems operated. The literature reports that the optimal growth temperature for rainbow trout in culture ranges from 13 to 15°C (Janhunen et al., 2016).

RAS–A system included four independent subsystems (S0, S1, S2 and S3), each consisting of a 3 m³ trout pond connected to two DWC hydroponic beds, each one having a volume of 0.3 L, that added provided a growing area of 6 m². Each subsystem also incorporated a submerged nitrifying biofilter filled with recycled plastic caps, where the biofilter transforms fish waste (ammonia) into nitrates, which are nutrients that can be assimilated by plants. In this way, it maintains water quality, prevents toxicity for fish, and ensures a balanced cycle between fish and plants. A radial sedimentation tank and a storage tank that received effluent water from the hydroponic beds.

Considering these four subsystems, three (S1, S2 and S3) were bio integrated aquaponic systems, with simultaneous cultivation of trout (*Oncorhynchus mykiss*) and lettuce (*Lactuca sativa*), being operated in parallel. The fourth subsystem (S0) served as a control system, that included only trout cultivation without the integration with plants (Fig. 35). A hydraulic pump, 1 HP, connected to a 1 m³ accumulator was used to recirculate water to the fish tanks.

Figure 36 illustrates the RAS-B system, that used a similar configuration and water dynamics than RAS - A. The main difference was that RAS -B included two trout tanks and four hydroponic beds, both operating simultaneously. In addition, in this system the individual water volume was lower, where each trout tank was 1 m³ equipped with two hydroponic DWC of 0.3 L each, with a total volume of 1,600 L and a hydroponic area of 6 m².

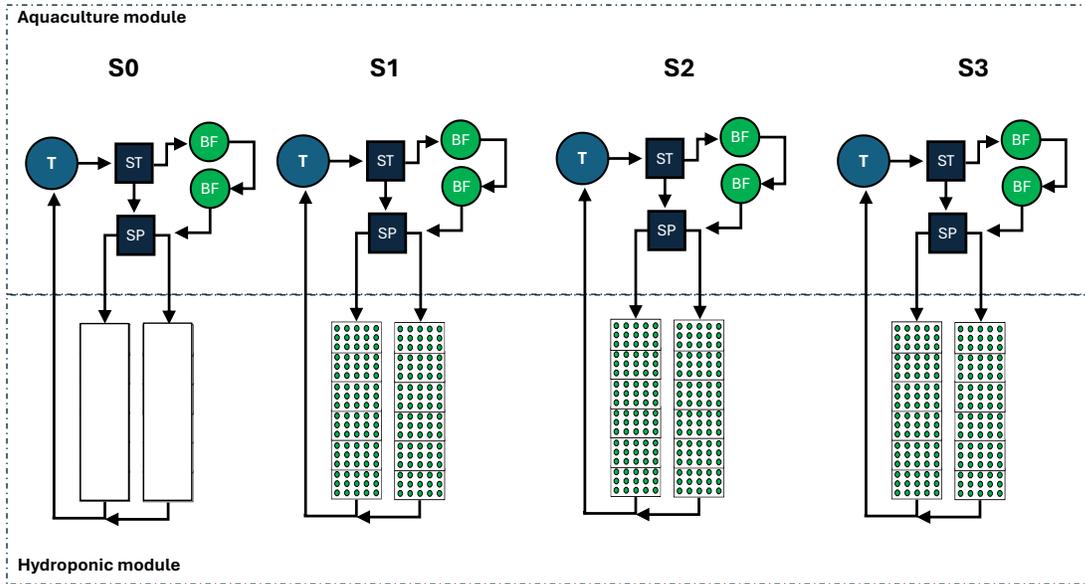
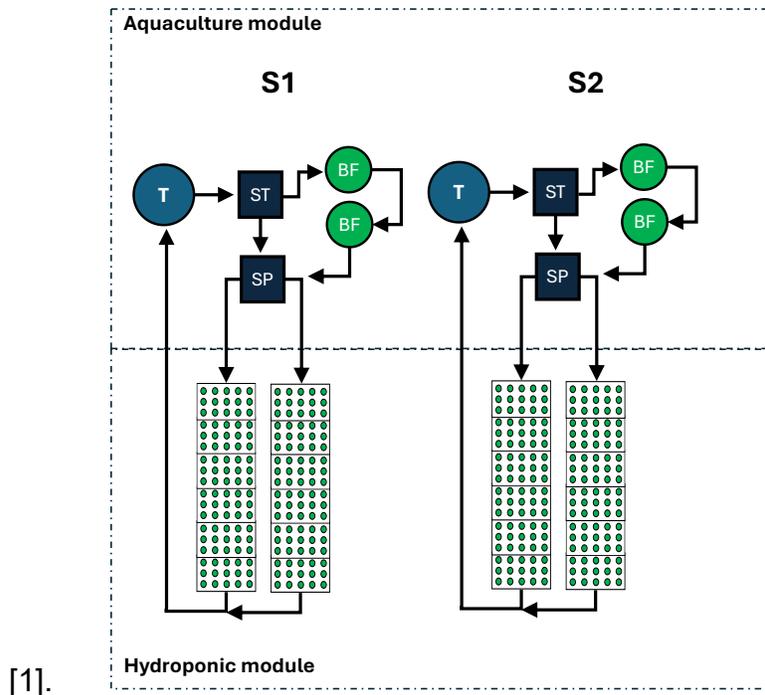


Figure 35. RAS – A schematic design, subdivided in four Aquaculture modules and hydroponics modules (S0, S1, S2 and S3). Where T: Trout tank, ST: Radial Flow Settler, SP: Sump, BF: Biofilter.



[1].

Figure 36. RAS – B schematic design, subdivided in two Aquaculture modules and hydroponics modules (S1 and S2). Where T: Trout tank, ST: Radial Flow Settler, SP: Sump, BF: Biofilter.

The systems were designed based on the mass balance procedure detailed below. The purpose of this analysis was to identify and quantify the inputs, outputs and internal changes (conversions and consumptions) of the farming system. This methodological approach, as pointed out by Kalvakaalva et al. (2023), is essential to obtain a comprehensive understanding of the system and contribute to the development of the required effective strategies to accomplish for efficient management of the productive system. In the field of scientific research, the implementation of this tool resulted in the ability to accurately identify the dynamics and transformation of the main components of the aquaponic system. Figure 37 depicts the overall procedure describing the mass flow, crop cycles, and nitrogen conversion for each aquaponic system.

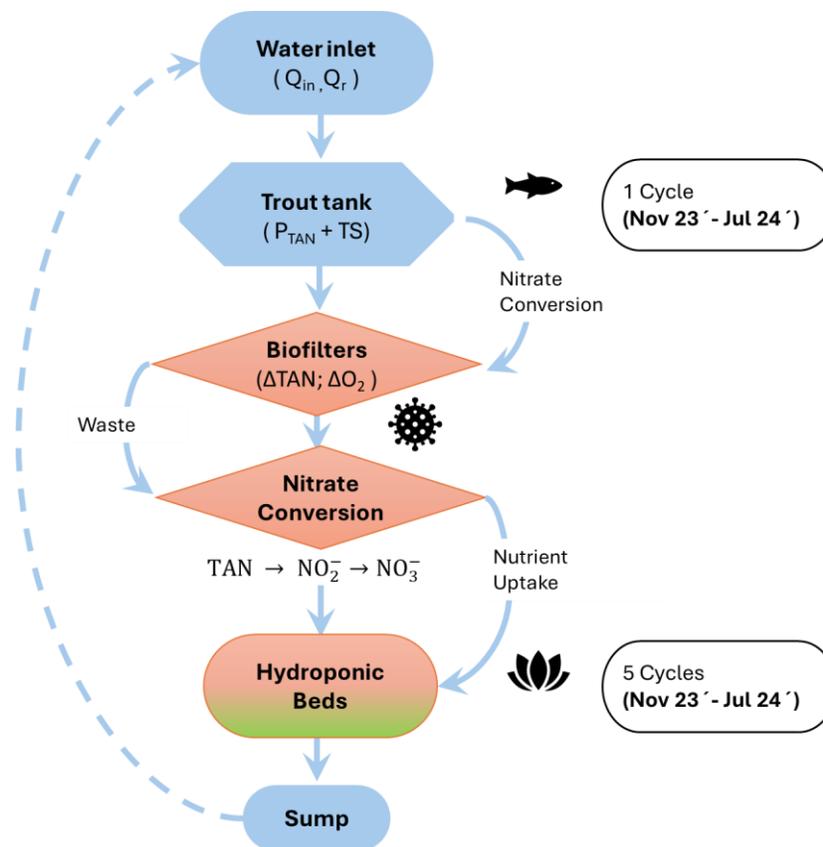


Figure 37. Mass flows in the trout, lettuce and treatment units of the aquaponics systems.

4.4.2 General Mass Balance

This general mass balance equation (Eq. 9) was used under the assumption of a steady-state process analysis (Timmons et al., 2002).

$$(Q_m \times C_m) + (Q_r \times C_r) + P = (Q_m \times C_{\text{tank}}) + (Q_r \times C_{\text{tank}}) \quad (9)$$

where Q_{in} is the inlet flow (L/min), C_{in} the inlet species concentration (mg/L), Q_r corresponds to the recirculating flow (L/min), C_r is the species concentration of recirculating flow (mg/L), P is the mass production (mg/min) and C_{tank} is the mass variation in the tank (mg/L).

The mass balance was carried out for the following components: Nitrate (NO_3^{-2}), Total Ammoniacal Nitrogen ($\text{NAT} = \text{NH}_3 + \text{NH}_4^+$), Dissolved Oxygen (DO) and Total Solids (TS); by means of the conversions and the assumptions stated in Table 10. DO levels were determined to maintain the growth in the fish and flow rate (Q) required for the entire biosystem and its water treatment units. The water quality parameters used correspond to those given by Wolters et al. (2009) and (Timmons et al., 1998) and are presented in a detailed way in Table 10.

The procedure followed for the mass flow analysis was based on the methodologies outlined by Rakocy et al., (1998) and Kalvakaalva et al. (2023). The analysis encompassed the following sections: water volume of culture, size of the biomass, natural mass balance, sizing of the biological filtration unit and the physical filtration unit, and the oxygen mass balance. As illustrated in Figure 37, each analysis incorporated a few variables that were considered in relation to each one of the treatment processes.

Based on the maximum capacity of the system and the maximum stocking density (kg/m^3), the maximum feeding rate (% fish body weight) was then determined.

4.4.3 Design parameters and water recirculation.

The calculation of the total subsystem control volume used the maximum fish biomass (kg) established in the biological design and the maximum culture density (kg/m³) reported for the species in the recirculating aquaculture systems (RAS).

$$T_V = \frac{B_{\max}}{D_{\max}} \quad (10)$$

where T_V is the total crop volume of the system (m³), B_{\max} is the maximum crop biomass at pre-harvest (kg) and D_{\max} is the maximum crop density (kg/m³).

Table 10. Physical and biological design parameters of the aquaponic system.

Parameter	RAS - A	RAS - B	Units
Tank depth	1.2	1.2	m
Diameter of the tank	1.5	1	m
Total system volume	14	7	m ³
Maximum fish biomass	120	60	kg
Feeding range % of body weight /day	2		daily
Temperature	18 ± 2	15 ± 9	°C
Dissolved oxygen	6-8		mg/L
Total solids	<10		mg/L
Non-ionized ammonium	<0.0125		mg/L
Total Ammoniacal Nitrogen (TAN)	<1		mg/L
NH ₃ -N	<0.02		mg/L
Nitrite-N	<0.1		mg/L

The mass balance was conducted through a comprehensive literature review concerning the biological and physical design parameters of each one of the subsystems. This analysis encompassed the culture density and water quality

parameters, specifically relevant to the culture of *O. mykiss*. The percentage of water recirculation was determined to be 97%, and equation 3 was applied accordingly,

$$R = \frac{Q}{Q + Q_0} \quad (11)$$

where R is the percentage of water recirculation (%), Q corresponds to the predominant flow rate of the system (highest maintenance flow rate; L/min), and Q_0 is the freshwater flow rate (make up water; L/min).

4.4.4 Biofiltration system design

The biofiltration system efficiently utilizes the metabolic waste produced by the fish, as well as uneaten food, transforming it into nutrients available for growing lettuce within the recirculating aquaponics system. From a chemical point of view, the central process is nitrification, whereby ammonium (NH_4^+) is oxidized under aerobic conditions. In the first stage, ammonium-oxidizing bacteria (AOB) convert ammonium into nitrite (NO_2^-); subsequently, nitrite-oxidizing bacteria (NOB) complete the sequence by transforming nitrite into nitrate (NO_3^-), the most stable form of nitrogen and the one most easily assimilated by plants.

4.4.5 Nitrification Mechanism in Aquaponics

Nitrification is one of the most critical processes for the stable and sustainable functioning of any aquaponics system. It is important because it acts as the biochemical link that transforms fish metabolic waste into nutrients that can be used by plants. Without nitrification, aquaponics could not be sustained as an integrated, recirculating system because it would fail to ensure the physiological safety of the fish and the effective nutrition of the plants.

In biogeochemical terms, nitrification is an aerobic process involving two functional groups of autotrophic bacteria: ammonium-oxidizing bacteria (AOB), which convert ammonium (NH_4^+) into nitrite (NO_2^-); and nitrite-oxidizing bacteria (NOB), which

transform nitrite into nitrate (NO_3^-). The latter is the most stable form of nitrogen: it is less toxic and more easily assimilated by most plant species. Therefore, nitrification establishes a metabolic bridge between fish and plants, converting nitrogenous waste generated by animal metabolism into essential nutrients for plant growth.

From an animal welfare perspective, nitrification is relevant because ammonium and nitrite are highly toxic to fish. In particular, non-ionized ammonia (NH_3) can cause physiological stress, gill damage, alterations in oxygen transport and mortality at concentrations above a few $\text{mg}\cdot\text{L}^{-1}$. Nitrite (NO_2^-), on the other hand, interferes with blood oxygen transport by forming methemoglobin, a condition known as 'brown blood disease'. Therefore, nitrification and biofiltration play an essential role in keeping these compounds within safe limits and ensuring the homeostasis of the aquatic environment in which fish live.

Nitrification is equally central for plants, as it determines the availability of nitrates, the main form of nitrogen used by fast-growing species such as lettuce. In aquaponic systems, nitrate produced in the biofilter compensates for the absence of external mineral fertilization, enabling competitive plant productivity to be maintained without synthetic inputs. Thus, nitrification forms the basis of internal nutrient recycling, enabling the system to function as a closed cycle in which nitrogen flows from fish feed to plant biomass.

In terms of system design, nitrification defines important parameters such as the size of the biofilter, the volume of the medium, the recirculation rates and the oxygenation requirements. An insufficient nitrification rate can lead to ammonium accumulation, decreased water quality and compromised system stability. Conversely, stable and adequately sized nitrification enables increased fish density, improved plant growth rates and maintained water quality, even under conditions of production intensification.

4.4.6 Mass balance of nitrogen compounds

In the design of recirculating aquaculture systems (RAS), the capacity to maintain acceptable levels of total ammoniacal nitrogen (TAN) in the culture water must be provided. To achieve this, the rate of TAN production and its nitrification to nitrate must be calculated (Hutchinson et al., 2004). For the present design, equation 12 was used with an assumption of feeding 2% of the biomass with a protein content of 48%.

$$PNAT = \frac{F \times PC \times 0,092}{T} \quad (12)$$

where PNAT is the total ammoniacal nitrogen production rate, kg/day, F the feed rate, kg/day, PC the protein concentration in feed, fraction and T is the period of time to feed a daily ration, days. Source: Timmons et al. (2002).

The Total Ammoniacal Nitrogen constant (TAN) was based on a series of approximations and estimates which, when multiplied, result in 0.092 (Eq. 13).

$$0.092 = 0.16 * 0.8 * 0.8 * 0.9 \quad (13)$$

where 16% is 48% protein with 16% nitrogen, 80% of nitrogen is assimilated, 80% of assimilated nitrogen is excreted, 90% of nitrogen is excreted as NAT and 10% as urea.

To maintain the desired concentration of N-NO₃ before the treatment unit, reduction by passive nitrification (assumed to be 10%) and passive denitrification (assumed to be 0%) were considered, thus obtaining the NAT concentration after both oxidations. After that, the maximum desired N-NO₃ concentration (defined in section 4.8) was considered and equation 11 was applied. In the case of the NAT concentration reaching the biofilter, the desired NAT concentration and the inlet flow rate for maintaining the desired N-NO₃ concentration were calculated using the NAT concentration after both oxidations.

4.4.7 Biofiltration unit sizing.

When designing the biofilter, consideration was given to the type of biological filter to be used, the surface nitrification rate (g NAT/m²/d) and the volumetric nitrification rate (g NAT/m³/d), as well as the types of substrate and their surface area, in order to ensure operation in cold water and for the selected fish species.

The mass flow of NAT before reaching the biofilter (point 4.9) and the NAT surface nitrification rate (g NAT/m²/d) were used as design criteria for nitrification to determine the required substrate surface area (Eq. 13). This corresponds to the area of the medium (substrate) required to fix autotrophic bacterial populations that oxidize ammonium to nitrite and then nitrate (Hutchinson et al., 2004).

The surface nitrification rate of NAT (g NAT/m²/d) was determined using the modified Michaelis–Menten (M-M) model (Equations 13 and 14), as described by Chen (2005), based on the NAT concentration at the biofilter inlet (mg/L) in a recirculating water system at 15 °C.

$$R = \frac{R_{\max}}{K_s} \times (S - 0,07) \quad (14)$$

where R is the Surface nitrification rate of NAT, g NAT/m²/d, R_{max} the maximum surface removal rate of NAT, g NAT/m²/d, S the concentration of NAT, mg/l, and K_s is the average saturation constant, mg/l. / (Chen, 2005).

Applying the values described by Chen (2005) for R_{max} (3.33 g NAT/m²/d) and K_s (5.3 mg/l) respectively, Eq. 14 is reduced to the following:

$$R = (588 * S) - 41 \quad (15)$$

Once the surface nitrification rate of NAT (g NAT/m²/d) was obtained, the support surface area for NAT nitrification was calculated (m²) (Eq. 15), and based on the surface area of the substrate (m²/m³) used in this design, the volume of support medium required for NAT nitrification (m³) was obtained from Eq. 16,

$$A_s = \frac{C_{NAT}}{R} * 1000 \quad (16)$$

where A_s is the support surface area for NAT nitrification, m^2 ; C_{NAT} the concentration of NAT reaching the biofilters, kg/d , and R the surface removal rate of NAT, $g\ NAT/m^3/d$ (Timmons et al. 2002).

$$V_s = \frac{A_s}{S_s} \quad (17)$$

where V_s : volume of support medium required for NAT removal, m^3 , A_s : support surface area for NAT removal, m^2 , S_s : surface area of the substrate, m^2/m^3 (Source: Hutchinson et al. (2004).

The biofilter reactor was designed using all the data calculated above as a tool.

4.4.8 Nitrification process modelling

To analyze the behavior of the biofilter within the aquaponics system, a dynamic model was constructed in Stella® that represents the total ammonia nitrogen (TAN) balance in the biological treatment unit (Fig. 38). The model was based on the classic principles of recirculating aquaculture system design, in which it is essential to maintain the total ammoniacal nitrogen concentration within safe ranges through the action of nitrifying bacteria.

In the flow diagram, the central state Total Nitrogen represents the total ammoniacal nitrogen content in the volume of water associated with the biofilter. This state evolves over time according to the difference between a nitrogen production rate associated with fish excretion ($Total_Nitrogen_prod_fish$) and a removal rate due to bacterial nitrification ($Total_Nitrogen_removal$). In discrete terms, the update equation used in Stella was expressed for the total amounts as:

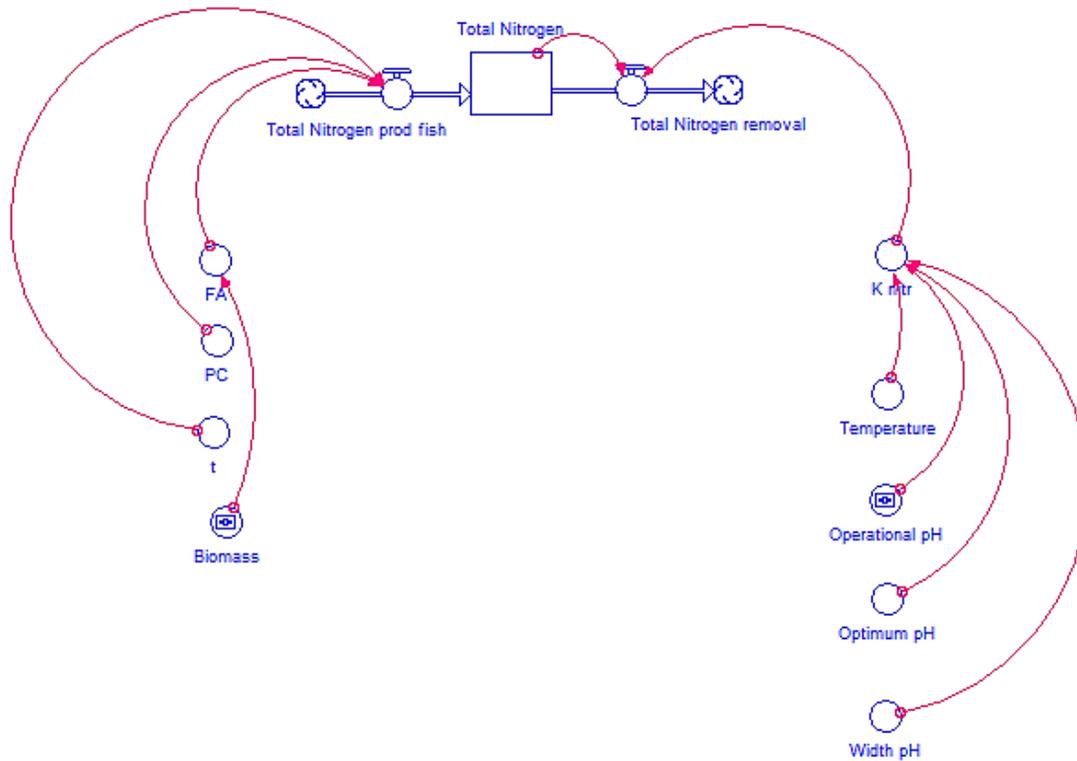


Figure 38. Stella® diagram of the nitrification process in biofilters.

$$\text{Nitrogen}(t) = \text{Nitrogen}(t - \Delta t) + (\text{Nitrogen Produced} - \text{Nitrogen removed}) \cdot \nabla t \quad (18)$$

where Δt is the numerical integration time step. The state was initialized with a reference value (Total_Nitrogen = 1), corresponding to a base TAN concentration from which the dynamic trajectories were analyzed.

4.4.9 Nitrogen production from food

Nitrogen production by fish was modelled based on the feeding rate and the protein fraction of the feed. In the model, fish biomass was initially set at 0.5 kg and the feeding rate (FA) was calculated as 2% of biomass:

$$\text{FA} = \text{Biomass} \cdot 0,02 \quad (19)$$

which is consistent with typical feeding strategies in cold water trout fattening systems. Based on this feeding rate, the assimilable nitrogen production was estimated to use a protein content of 40% in the feed (PC = 0.4) and an overall conversion factor from protein to excreted nitrogen. In Stella, the total nitrogen production rate by the fish was expressed as:

$$\text{Total Nitrogen Produced} = \frac{(FA \cdot PC \cdot 0.102)}{t} \quad (20)$$

where t corresponds to the reference period (in this case, 3 days). The coefficient 0.102 groups together a series of approximations widely used in the design of recirculating systems: fraction of nitrogen in protein, assimilation efficiency and proportion of nitrogen excreted in the form of TAN.

In this way, the model explicitly links the nitrogen load entering the biofilter with management decisions such as crop biomass, feeding rate, and feed nutritional quality.

4.4.10 Nitrogen removal by nitrification

The nitrogen removal rate was represented by a first-order term dependent on the TAN concentration and an effective nitrification constant:

$$\text{Total Nitrogen removal} = k(n) \cdot \text{Total Nitrogen} \quad (21)$$

where k(n) is the nitrification constant adjusted to the environmental conditions of the system. This constant was not considered fixed but was made explicitly dependent on water temperature and operating pH, in accordance with the experimental evidence indicating that both factors strongly modulate the activity of nitrifying bacteria.

4.4.11 Total modelled TAN

In Stella's model, the nitrification constant $k(n)$, was defined by a relationship combining a linear effect of temperature with a logistic term that penalizes deviations from the optimum pH for nitrification:

$$k(n) = \frac{0.01 \cdot (T-20)}{1 + \exp \frac{pH_{op} - pH_{opt}}{width\ pH}} \quad (22)$$

Here, T corresponds to the water temperature (°C), initially set at 20°C; pH Op is the actual pH of the system (e.g., 6); pH opt represents the optimum pH for nitrification (in this case, 8); and width pH is a sensitivity parameter (0.2) that controls how quickly nitrifying activity decreases when the pH deviates from the optimum value. Thus, when the temperature rises above 20°C and the pH approaches its optimum value, when nitrification constant increases TAN removal intensifies; conversely, at suboptimal pH values, the constant decreases, reflecting slower nitrification.

This approach made it possible to capture in a single expression the combined effect of temperature and pH on biofilter efficiency, integrating experimental knowledge about the sensitivity of ammonium-oxidizing and nitrite-oxidizing bacteria to these variables.

4.4.12 Biofilter sizing linkage

The Stella dynamic model was complemented by the classic biofilter sizing scheme used in recirculating aquaculture systems. Based on the total ammoniacal nitrogen production rate (TANPR), calculated according to feed and feed protein content, the mass flow of TAN entering the biofilter was estimated.

The surface nitrification rate was then determined using a modified version of the Michaelis–Menten model, based on the TAN concentration at the biofilter inlet and kinetic parameters (R_{max} and K_s) specific for cold water systems. This rate was used to calculate the support surface area required for nitrification, and the specific area of the medium (m^2/m^3) was used to obtain the volume of support medium required.

Overall, the model implemented in Stella allowed for an explicit representation of the relationship between: (a) the nitrogen load generated by fish biomass and feed, (b) the removal capacity of the biofilter modulated by temperature and pH, and (c) the criteria for sizing the support medium.

4.4.13 Settler tank

The Radial Flow Settler tank volume was estimated from the experience of (Summerfelt & Penne, 2005). Assuming a solids removal fraction of 27% by lateral drainage, and 20% of Total Suspended Solids (TSS) removal efficiency. The remaining TSS leaving the settler went directly to a mineralizer. The calculation of the surface area required for sedimentation and the diameter of the settler was based on the use of equations 22 and 23, respectively.

$$A_{ZS} = \frac{Q_{IN}}{V_0} \quad (23)$$

where A_{ZS} is the area required for sedimentation (m^2), Q_{IN} the inflow to the settling tank (m^3/h) and V_0 is volumetric flow rate per unit area or the overflow rate of the settling tank ($m^3/h/m^2$). The upper diameter of settler tank (φ_s) was obtained from,

$$\varphi_s = \sqrt{\frac{4 * A_{ZS}}{\pi}} \quad (24)$$

The other settler dimensions considered a design factor based on the quotient between the surface areas required for sedimentation (m^2) of the present design and that described by (Davidson & Summerfelt, 2005). This factor ($f = 1.18$) was then applied to the dimensions of settler height (h) and cone height (h_c) described in the same work. In the case of the lower cone diameter (φ_l), the upper to lower diameter ratio (31.5:1) of the (Davidson & Summerfelt, 2005) design was used, assuming the same value of 60° for the cone inclination.

Aeration in each system was provided by a Sweetwater 3 HP blower. Each aquaponic bio-integrated system was connected to a 24,000 BTU heat pump, which allowed a stable temperature of 20°C to be maintained throughout the cycle, considered optimal for trout culture. The system operated with a daily 5% make-up water replenishment, ensuring adequate conditions for the development of the organisms (Fig. 39).

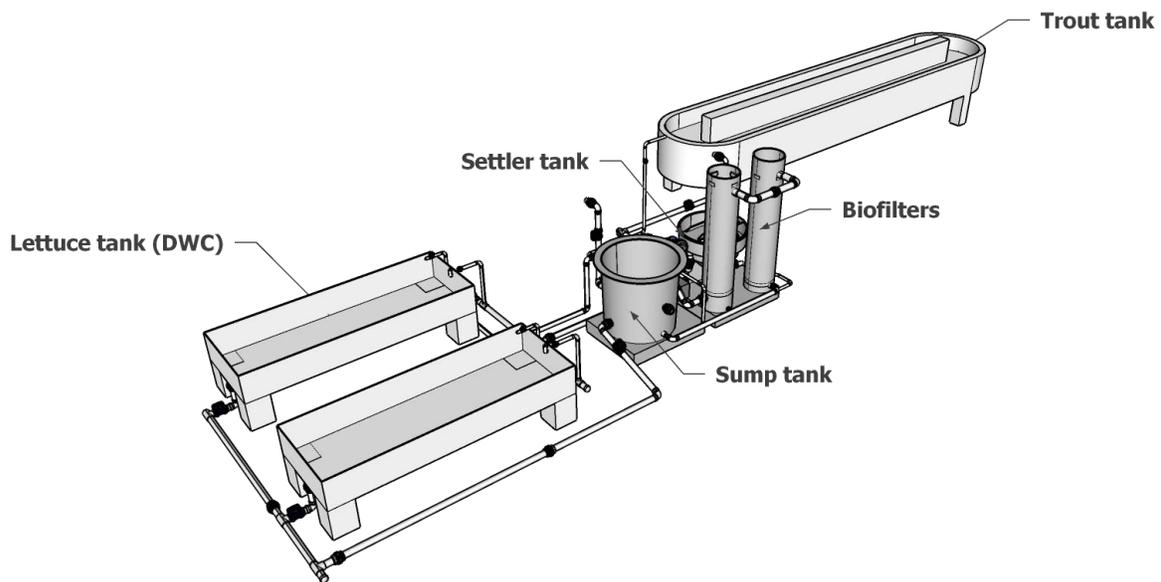


Figure 39. Aquaponics facilities at Laboratorio de Bioingeniería en Acuicultura (Facultad de Ciencias del Mar, Universidad Católica del Norte). One unit consisting of one trout tank, two biofilters, one settler tank, sump and two hydroponic DWC beds, where RAS- A and RAS- B have the same configuration.

In RAS – A all three systems incorporated fish and plants in a coupled mode (S1, S2 and S3), maintaining a final density of 30 kg/m³ in all tanks, except for the control S₀ System, which was never coupled to the hydroponic beds. In contrast, in RAS - B both systems, S1 and S2, operated in the same way.

Using the rule of Rakocy (2007), for every 60 g of feed, 1 m² of vegetables was supported. Therefore, fish were established first and once 60 g of feed was supplied.

From then on, vegetable planting was carried out with a density of 25 plants/m². Recommended stocking density in aquaponics systems integrating trout and lettuce should balance efficient production with animal welfare and system integrity. For *O. mykiss*, a stock density between 20-30 kg/m³ is suggested, depending on the recirculation system and water oxygenation level, to avoid stress and health problems, (Fabula et al., 2023; Kitaya et al., 2023).

4.5 Results and discussion

4.5.1 Lettuce performance

Plant performance for RAS – A and RAS – B was assessed for Fractional Green Canopy Cover (FGCC), root and leaf growth, and stem diameter, using as a reference morphometric and productive variables reported by (Cortés et al., 2024), as illustrated by the schematic view in Figure 40.

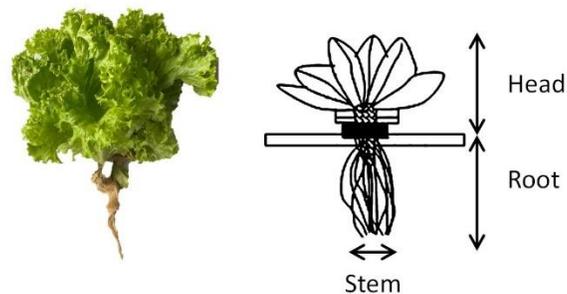


Figure 40. Head, root and stem measured zones of lettuce (Modified from Wang et al., 2021), actual picture is shown on the left.

The FGCC was measured once a day at 10:00 AM, with a frequency of twice a week, using digital photographs via the Canopeo app ®. This method provided a consistent and efficient way to monitor vegetation cover over time, ensuring accurate tracking of the vegetation changes. Root and aerial height were measured weekly using a digital caliper of accuracy ± 0.03 mm. Measurements were taken from the base of the plant to the point of greatest height. This approach allowed the precise monitoring of plant growth, ensuring uniformity in the data collection process (Cortés et al., 2024).

At harvest time, three trays were selected for measurement, one from each end and half from each hydroponic bed, with a quantity of 45 plants per bed. For partial transplanting cycles, the entire crop was harvested to avoid inconsistencies in the data collection. This sampling strategy increased the representativeness of the data, ensuring the accurate capture of growth variations throughout the system, as depicted in Figure 41.

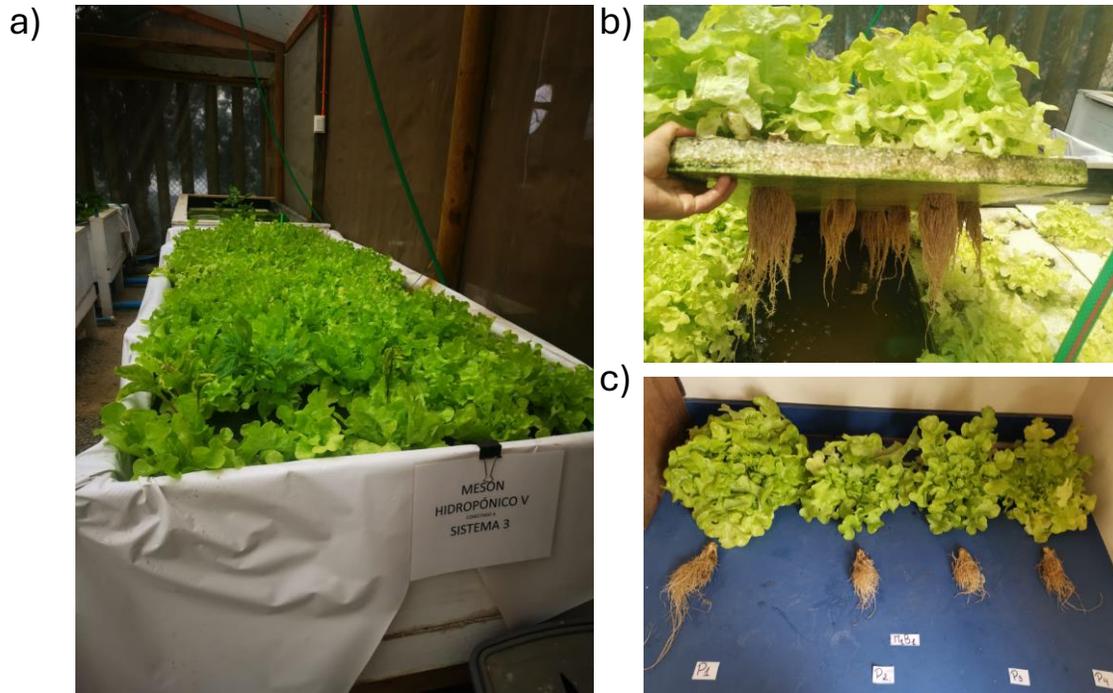


Figure 41. Growth and harvesting lettuce (a), harvesting (b), and measurement of lettuce morphometric variables (c).

4.5.2 Trout growth

Fish growth in RAS -A, for systems S1, S2 and S3 exhibited an increasing trend over time, with some systems standing out more than others. Trout commercial diet for RAS – A and B consisted of EWOS ® formulated feed of variable size for each growth stage, starting at size 2 and ending at size 6. The feed supply in each system was 2 to 3% of body weight per day, divided into two portions.

Figure 42 illustrated that from the sixth month on, the differences between the systems started to increase, with S3 showing the highest growth in weight towards the end of the period, exceeding 600 g per fish in month 9. S3 experienced a more accelerated growth compared to the other subsystems. The S1 and S2 systems, although showing a lower pronounced growth than S3, also exhibit a monotonous increment in the fish weight, reaching around 500 g at the end of the period. Growth in these systems was more gradual and uniform, as depicted in Figure 42.

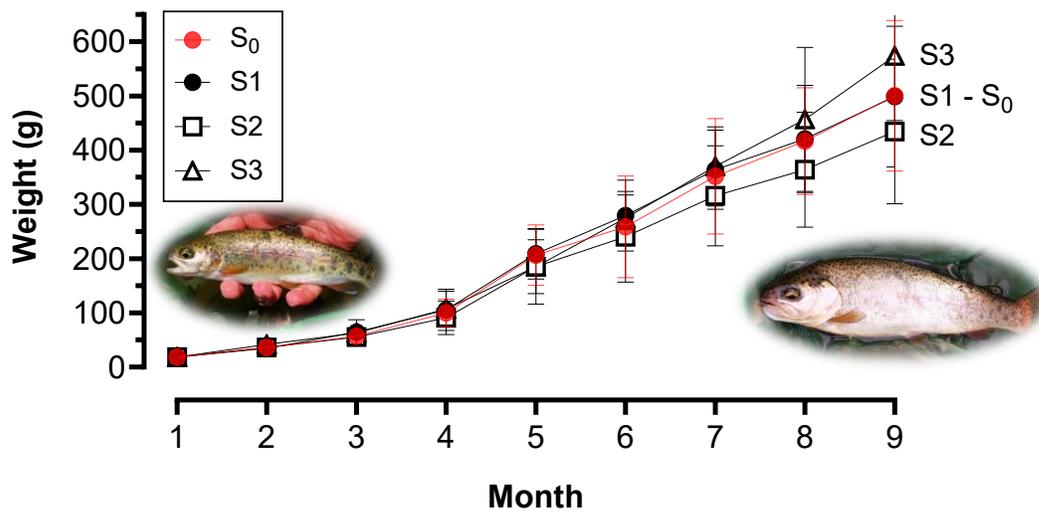


Figure 42. Trout growth curve for the system with only fish system (S0), and lettuce-systems 1 (S1), system 2 (S2) and system 3 (S3), between November 2023 to July 2024.

Fish growth is directly related to Nitrate levels in the water, as their excretions have been found to the generation of nitrogenous compounds that are transformed into nitrate (Schmautz et al., 2021). In S1, the steady fish growth coincides with a significant increment in nitrate, while in S3, despite the faster fish growth rate, Nitrate levels did not increase dramatically, suggesting that the system had a greater capacity to handle the nitrogenous waste, as illustrated in Figure 43.

When comparing the monthly growth of both experiments it can be observed that the fish reared in the RAS - A, under controlled internal temperature conditions, reached a final weight close to 600 g in the month 9 (treatment S3), while in the system at

ambient temperature (RAS - B) the maximum values were in the range between 450 g and 500 g for the same period. The differences became more evident from month 6 onwards, where in the RAS -A, with treatments S1 and S3 the trout weight exceeded 300 g, while in the system without temperature control, the increment was more gradual, with lower weight gains in the same time interval, as depicted in Figure 43. Quantitatively, the implementation of water thermal control allowed to achieve cumulative growth rates up to 20–25% higher towards the end of the cycle. Furthermore, trout weight exhibited a lower dispersion during the initial months in the system with the controlled water temperature and a higher growing rate in the final fattening phase, suggesting a more efficient use of feed and better metabolic conditions in a uniform temperature environment. The average water temperature in RAS -A was $18 \pm 2^{\circ}\text{C}$, and in RAS-B was $15 \pm 9^{\circ}\text{C}$. In both systems of RAS – B, S1 and S2, the observed monthly growth of trout was similar.

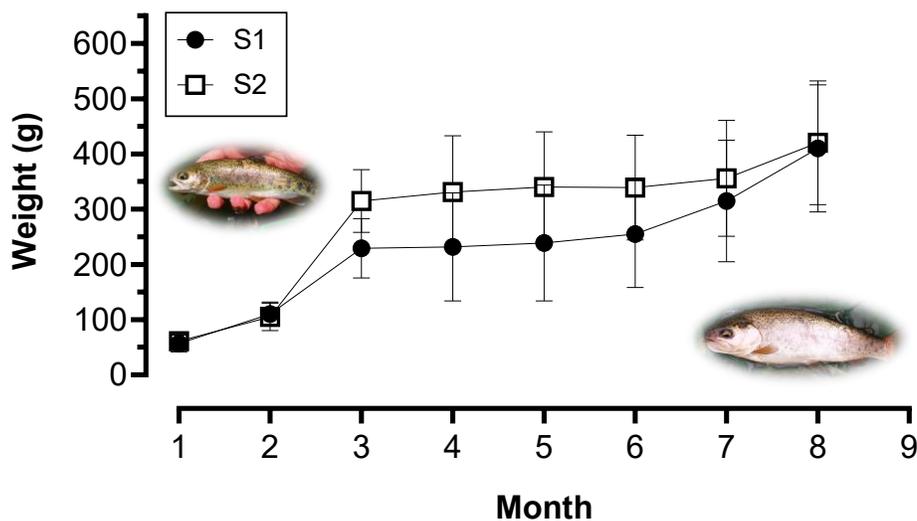


Figure 43. Evolution of trout weight for the RAS -B system, between November 2023 to July 2024.

Growth of *O. mykiss* in a closed aquaponic system was sustained and efficient, with final weights close to 600 grams over a period of nine months. Cubic fitting curves used to model growth show an excellent fit, with coefficients of determination (R^2)

between 0.995 and 0.998 and root mean square error (RMSE) values lower than 11 g, indicating a high reliability of the model to describe the observed growth pattern.

When comparing these findings with previous studies, a similar trend was observed for the trout growing rate, but with certain advantages in favor of the present aquaponic system used. Janampa-Sarmiento et al. (2020), reported a growth of trout fed with vegetable diets, reaching weights between 300 and 450 grams in a period of 90 to 120 days, showing specific growth rates in the order of 1.2 to 1.4%/day. Although (Zhao et al., 2023) considers a traditional intensive system, the reported results show specific growth rates of approximately 3.1%/day in rainbow trout, achieved under controlled water temperatures of 12–14 °C and low culture densities (3-10 kg/m³). The specific growth rates were calculated as follows (Eq. 25).

$$SGR = \frac{\ln(W_t) - \ln(W_0)}{\Delta t} \times 100 \quad (25)$$

where W_0 is initial weight, W_t is final weight, and t time elapsed between measurements, for each system.

In comparison, the recirculating system evaluated in this study, which incorporates nutrient uptake by plants, achieved equal or even higher yields, with RAS-A reaching specific growth rates of approximately 1.1%/day and RAS-B around 0.6%/day.

The polynomial trout growth model applied in this study is similar to the one proposed by (Parker et al., 2011), where the trout growth was evaluated using cubic regressions under different environmental conditions. In that work, high R^2 values (>0.98) were also achieved. However, the present aquaponic system, with temperature control, that included partial water replacement and integrated biofiltration, allowed for an even more accurate fit and a smoothly accelerated growth curve towards the last months of the cycle (Fig. 44).

The analysis of the trout growing results for the aquaponic system without lettuce, the S_0 group, indicated a growth pattern initially comparable to that of the hybrid trout-lettuce systems, but showed a greater dispersion in weights and a more irregular growth curve during the advanced stages.

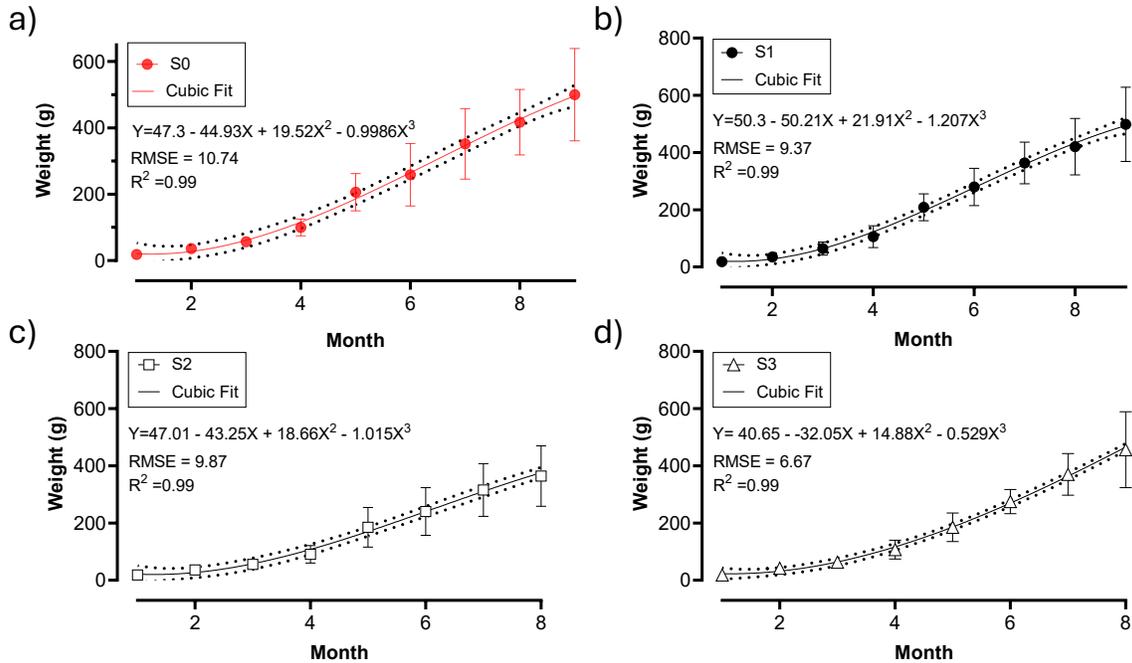


Figure 44. History of trout growth for the only fish system, comparison of actual growth data versus the fitted cubic model, for S₀ (a), System 1 (a), System 2 (b) and System 3 (c), cubic fit, R² and Root Mean Square Error (RMSE) for each system. The dotted lines represent the 95% confidence bands of the fitted model.

Despite reaching a final weight close to 500 g, the increment in the standard deviation suggested a less suitable environment for fish development. This condition can be attributed to the accumulation of metabolites such as ammonium and nitrite, a product of an incomplete nitrification cycle in the absence of plant extraction. The lack of plants prevented the effective assimilation of mineralized nitrogen, disrupting the microbial balance and affecting the water quality required for improved trout growth. In contrast, systems S₁, S₂ and S₃ - where there was a functional connection with plant crops - showed more sustained growth rates with lower variability, because of achieving a more biochemically balanced environment.

RAS-B, which was a system without thermal control that was exposed to the environmental variability of a semi-arid climate, shows a larger data dispersion and uncertainty (wider error bars), as well as more erratic growth (Fig. 45), compared to RAS-A, which has thermal control. While RAS-B generally follows an upward trend,

the rate of weight increase was lower and less predictable than the one found in the RAS-A system.

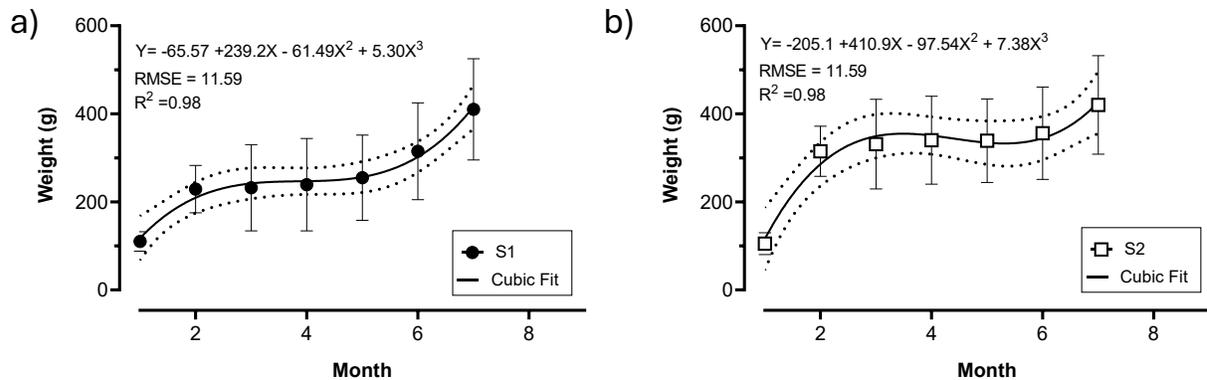


Figure 45. Evolution of trout weight for RAS – B. Comparison of actual growth data versus fitted cubic model, R^2 and Root Mean Square Error (RMSE) for System 1 (a) and System 2 (b). The dotted lines represent the 95% confidence bands of the fitted model.

The results of growing rate of trout in the hybrid system with water temperature control reinforce the notion that trophic integration between fish and plants in aquaponics not only improves the nutrient utilization but also contributes significantly to the physiological and productive stability of the trout-lettuce integrated system.

To assess the differences in the variability of trout growth among RAS-A and RAS-B subsystems, a simulation-based approach was employed. For each subsystem, the data of individual trout weight were adjusted using a normal distribution parameterized by the reported monthly means and the standard deviations. Ten replicates per month were generated to approximate the natural variability observed under real farming conditions. These simulated datasets were then used to test the homogeneity of variances across subsystems. Specifically, three statistical tests were applied: Levene's test, Bartlett's test, and the Fligner-Killeen test, the latter being more robust to data with deviations from normality (Pallmann et al., 2014). Additionally, pairwise comparisons between each subsystem were conducted using the F-test, allowing for direct assessment of the variance differences.

A comparison between the predicted evolution of the trout weights achieved between the aquaponic systems RAS-A and RAS-B reveals significant differences in both predicted weight over time. In RAS-A, which operated under controlled temperature conditions, all trout subsystems demonstrated consistent monthly increment in the trout weight. However, a larger variation was observed in subsystems S0 and S3, as indicated by their wider standard deviations (Fig. 46).

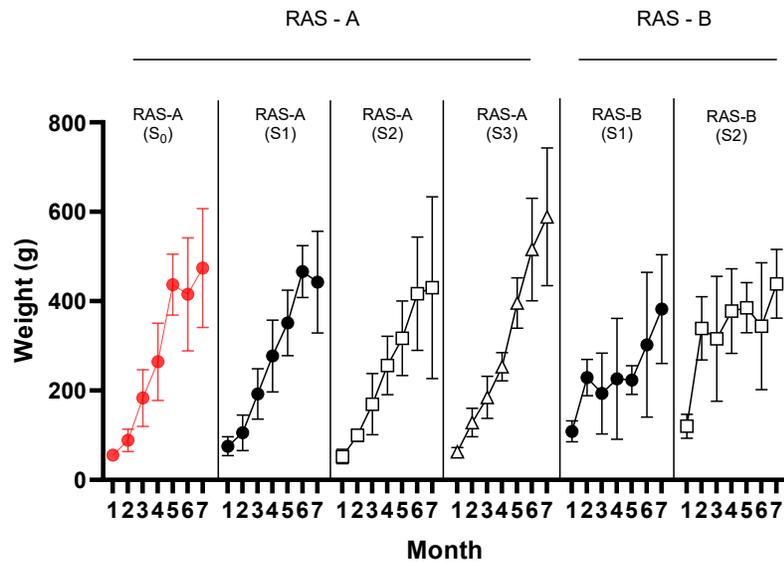


Figure 46. Prediction of monthly trout weights, for each system and subsystem.

This dispersion suggests the presence of heterogeneous growth conditions, possibly arising from biological variability or from systemic factors such as nutrient accumulation. In contrast, subsystems S1 and S2 exhibited noticeably tighter error bars, particularly during the later months of the production cycle. This stability is likely attributable to the combined effect of the constant thermal regulation and the integration between trout and plants, which may contribute to a more balanced nutrient dynamic.

Subsystem S0, which lacked plant integration, displayed the highest variability within RAS-A, reinforcing the hypothesis that the absence of plant uptake mechanisms can lead to fluctuations in the nutrient concentrations—particularly of nitrogen and calcium—that in turn affect the uniformity in the fish growth.

In the case of RAS-B, the trout subsystems also followed a pattern of weight increase over time, but the trout population started with comparatively higher initial weights. This aligns with previously noted observations of a higher initial biomass (+80%) in this system. Nevertheless, as the months progressed, trout weights in RAS-A began to match or even surpass those in RAS-B. This trend reversal suggests that, while RAS-B may offer a strong initial weight in terms of biomass accumulation, RAS-A environmental stability ultimately supported a more sustained and consistent trout growth.

Furthermore, the variability in trout weights within RAS-B remained high throughout the study period. This elevated dispersion is plausibly linked to the ambient temperature regime under which the system operated, which could have introduced fluctuations in key water parameters such as dissolved oxygen and unexpected changes in the thermal profiles. These environmental swings likely affected the metabolic rates and feed conversion efficiency of the trout, leading to less predictable growth outcomes.

The analysis of the evolution of the trout weight by the error bar distribution across systems supports the statistical analyses conducted on variance homogeneity. The RAS-A system allowed to achieve more uniform growth patterns, especially in subsystems S1 and S2, whereas RAS-B showed greater variability and less predictability.

4.5.3 Water quality and nutrients dynamics.

Towards the end of the experimental period, the S3 system showed a significant decrease in pH, which coincided with a drastic increment in the calcium concentration. This relationship suggests that as the availability of Calcium in the system increased, the acid-base balance also shifted, causing a decrease in pH. While the other systems exhibited a similar trend in the calcium/pH ratio, they did not experience such a large increment in the Calcium concentration, allowing to achieve more uniformity in the pH values that were maintained over time. This dynamic

evolution of pH observed in S3 can be explained by a more complex interaction between the metabolism of the system and its buffering capacity.

Indeed, the analysis of the trout growth data show that the system S3 also achieved the highest average trout weight, implying higher feeding rates and excretion of nitrogen compounds and minerals such as calcium. This behavior is consistent with that reported by (Tawaha et al., 2020), who indicated that higher fish densities in integrated aquaponic systems may accelerate nutrient accumulation and alter the chemical balance of the water. Furthermore, the study performed by Ngien et al., (2022) highlights the issue that in closed systems, small fluctuations in the chemical composition can trigger abrupt changes in pH if alkalinity being unable to buffer such changes, as appears to have occurred in S3. Goddek et al., 2016, argued that nutrient accumulation in coupled systems can lead to imbalances if plants are not able to efficiently absorb all the available compounds.

With regard to nitrate concentrations, the S1 system showed a considerable increase between January and February, without a parallel increment in the calcium concentration. This behavior suggests that, at least in this system, Nitrate was not directly related to the significant variations of found, that can be attributed to the presence of an effective buffering mechanism capable of maintaining the stability of the system by increasing the Nutrient concentration. This self-regulating capacity has been documented by (Schmautz et al., 2021), who pointed out that in well-balanced aquaponic systems, the progressive increment of nitrate due to nitrification does not necessarily lead to changes in pH, as long as there is sufficient alkalinity and plant uptake capacity.

In contrast, the S3 system showed in Figure 47 a simultaneous rise in calcium and nitrate, accompanied by an abrupt drop in pH, suggesting a more complex interaction between these factors. In this context, Yep & Zheng, 2019 suggested that the simultaneous accumulation of macronutrients can lead to unstable chemical conditions, especially in closed systems where recirculation is constant and plant roots, together with microbial processes, exert a direct influence on the water chemistry. The combination of these elements - calcium, nitrate and pH decrement

- observed in system S3 reflects the sensitivity of coupled aquaponic systems to imbalances between the rate of nutrient uptake and accumulation. This highlights the need for careful system management, where constant monitoring and adjustment of the relationship between nutrient input, biological activity and physical-chemical water conditions are the key factors to avoid imbalances that may compromise the aquaponic system stability and performance in the lettuce-trout growth process.

4.5.4 Lettuce growth

From November to January, pH of the three systems remained relatively constant at a value around 8. However, in February, a sharp drop in pH was observed in the S3 system, which seems to be affecting key aspects of plant growth in this system. Although S1 and S2 maintained a stable value of pH, the drop in S3 could be reflected on the plant stability and the development observed.

Leaf and root length evolution provided additional evidence on the performance of the systems. In both S1 and S2, sustained leaf growth was observed throughout the five harvest cycles (H1 to H5), which could be related to chemical water stability and higher nutrient uptake efficiency. In contrast, although plants in the S3 system also developed leaves, growth was less pronounced and more erratic, suggesting a less favorable environment for vegetative development. This pattern could be explained by the drop in pH, which, according to Monsees et al. (2019) can alter the bioavailability of key nutrients such as phosphorus and calcium, affecting root uptake and reducing the photosynthetic efficiency of plants.

Regarding root development, figure 48 reveals that S1 and S2 showed a more stable behavior with longer average lengths than S3. This last one with shorter roots and less consistency between cycles. According to Tunçelli & Memiş, (2024), root health in aquaponic systems is directly related to the pH balance, the oxygenation of the medium and the dynamics of nutrient exchange between the water and the root zone.

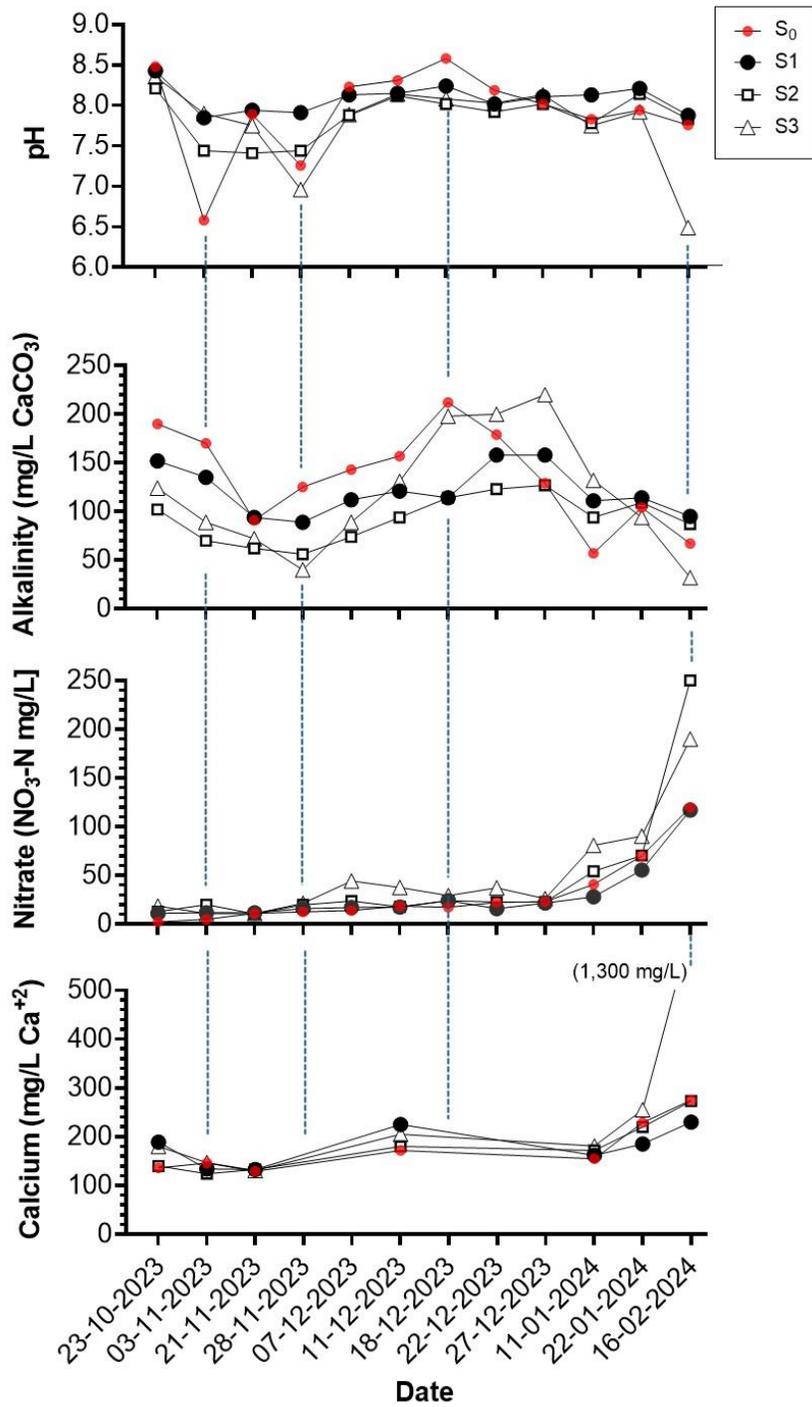


Figure 47. Evolution of pH, Calcium and Nitrate in RAS - A system (S₀, S₁, S₂, and S₃).

Therefore, the pH reduction observed in S3, coupled with some possible interferences in the assimilation of calcium or nitrate, could have limited the development of the root system and thus the ability of the plants to efficiently absorb the nutrients, as can be observed in Figure 48.

However, when comparing the three hybrid systems, it is observed that S1 and S2 maintained a balanced development in terms of leaf and root length, originating more favorable conditions for plant growth. In contrast, S3, affected by the drop in pH and lower root development, did not have an optimal performance. This interrelated behavior between pH, leaves and roots highlights the importance of maintaining high control of the system conditions to ensure efficient plant growth.

By contrast, plant growth in RAS-B was more consistent than in RAS-A, with a standard deviation between beds that was around 25% lower. However, the biomass achieved was 10% lower. Finally, the relationship between leaf and root length remained consistent throughout the cycles in both systems, responding strongly to their respective pH dynamics (Fig. 49).

4.5.5 Biofilter performance

Analysis of fish growth in relation to pH and alkalinity revealed significant differences between the four systems studied. The S3 group, which had the highest final weight (573 g), maintained stable and elevated values of pH throughout most of the trial, together with a progressive recovery of alkalinity, especially in January, reflecting a chemically favorable environment for fish growth. In contrast, the S₀ trout system, operated without plants, showed a sustained decrease in alkalinity and pH fluctuations towards the end of the cycle, suggesting a lack of biogeochemical regulation associated to the absence of plant uptake (Fig. 50).

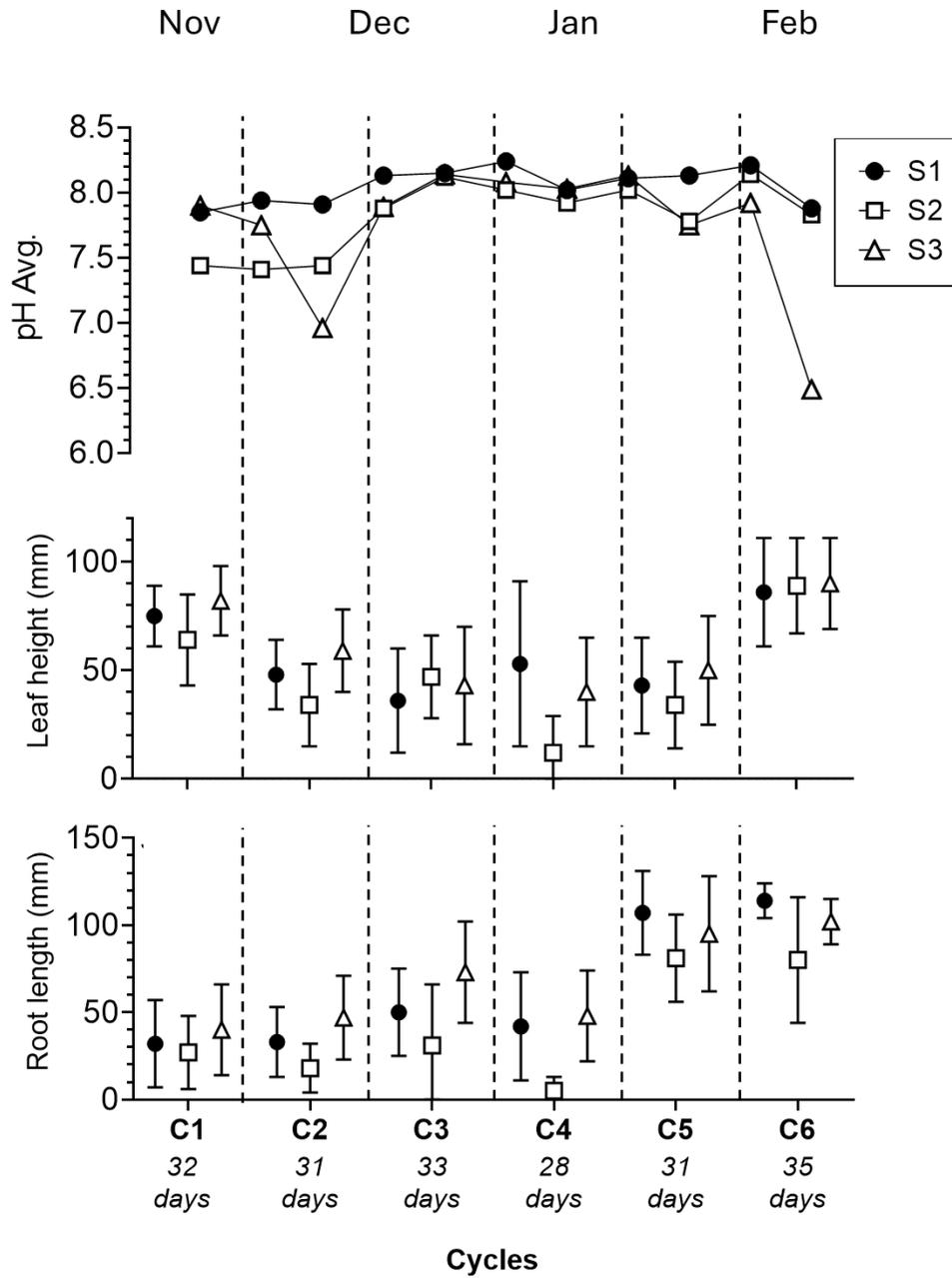


Figure 48. Effect of pH on leaf and root growth of lettuce in RAS-A aquaponic system.

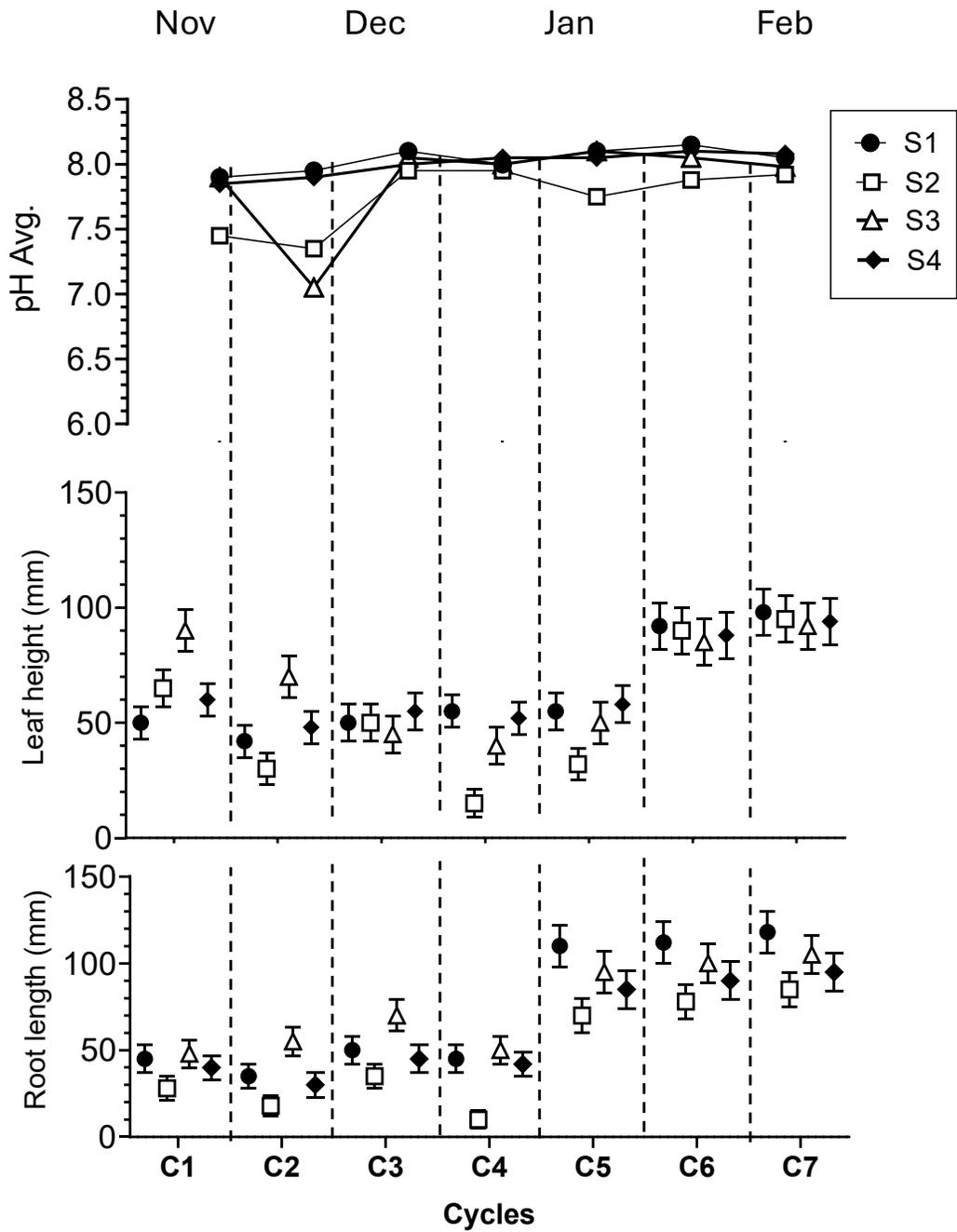


Figure 49. Effect of pH on leaf and root growth of lettuce in RAS-B aquaponic system.

On the other hand, groups S1 and S2 showed relatively stable pH values, but with lower and oscillating alkalinity levels, especially in S2, which may have partially limited the efficiency of the system and the growth achieved by the fish.

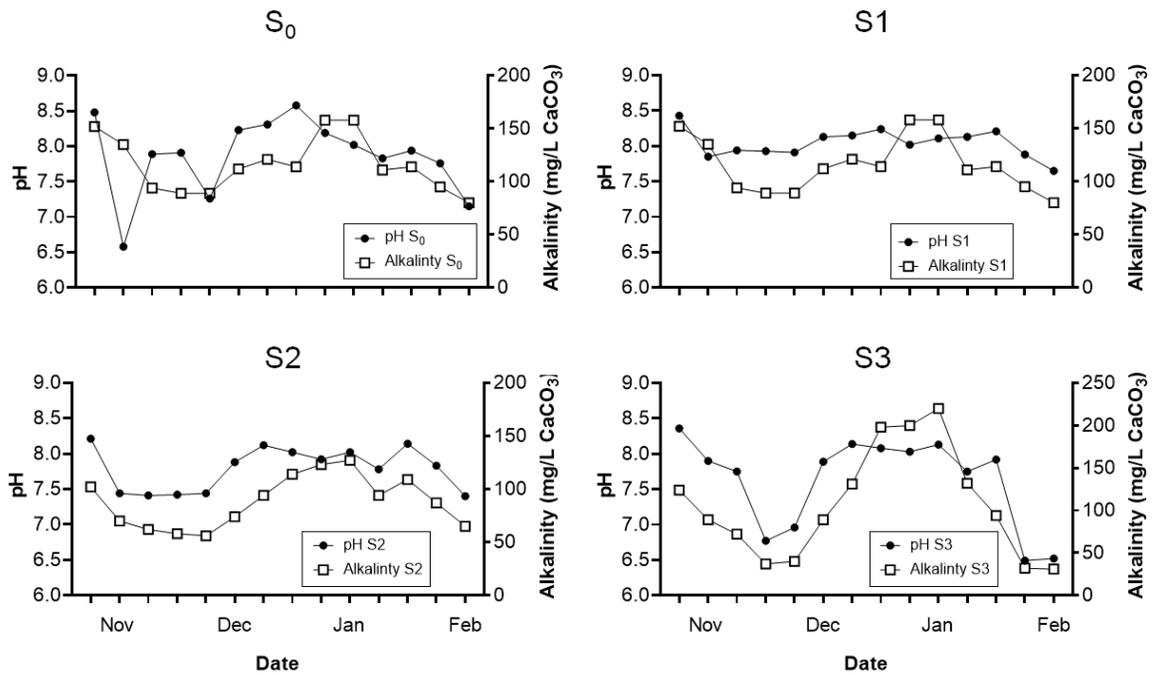


Figure 50. Relationship between pH and alkalinity in water for RAS-A system.

Regarding nitrogen dynamics, Figure 51 depicts that the differences between treatments were also marked. The S0 system showed a progressive accumulation of nitrite (NO₂-N) between December and January, reaching levels above 7 mg/L, without a proportional increment of nitrate (NO₃-N), indicating incomplete nitrification probably due to the lack of nitrate consuming plants that stabilized the cycle. In contrast, the S3 system revealed a progressive conversion of NO₂- to NO₃- over time, reflecting a more efficient nitrification process and an improved integration between the system components. The system S1 also managed to complete the nitrogen cycle, although it showed a marked peak of NO₂- before the accumulation of NO₃-, while in S2 high concentrations of both compounds were observed, with an

extreme value of nitrate close to 306 mg/L in February, suggesting a nutrient overload not well assimilated. These differences in nitrogen dynamics are directly related to the efficiency and stability of each system, and explain, in part, the contrasts observed in fish growth.

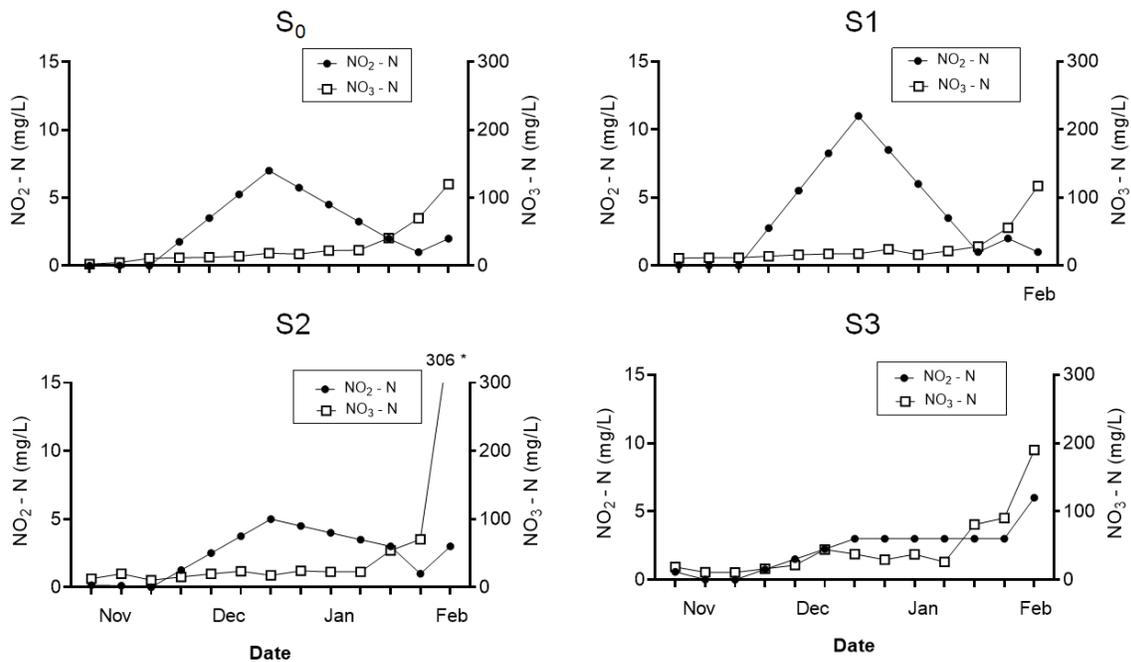


Figure 51. Relationship between $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ in water, for RAS-A system.

4.6 Discussion

This study explores the effects of macro and micronutrients, naturally generated by rainbow trout, in two aquaponic systems, and how the evolution of water quality in these systems influences the synergic development and growth of trout and lettuce in a Closed-Loop Aquaponic System.

The integration of fish and plants in aquaponic systems represents an efficient strategy for nutrient recycling, allowing water quality to be maintained and promoting balanced growth of both species. Our results show that coupled systems (S1 and S2), where trout and lettuce were integrated, exhibited greater stability in the values

of the key parameters such as pH, calcium, and nitrate concentration compared to the uncoupled system (S0) and the coupled system without plants (S3), which favored a more sustained plant development. This is consistent with the findings of Yamane et al. (2021) who concluded that aquaponic systems promote faster plant growth and maintain a better water quality than separate hydroponic or aquaculture systems.

Although in our study the initial growth of trout was faster in the decoupled system, the stability of the aquatic environment in the systems with plants provided clear benefits in the lettuce yield, particularly in systems S1 and S2. The water and nutritional balance have been highlighted in other studies, such as in those studies reported by Atique et al., (2022) and Bakhsh & Chopin, (2011), who conclude that maintaining adequate nitrogen and phosphorus levels is crucial to achieve the required synergy between fish and plants for successful growth.

Although some studies have shown that nutrients derived from fish do not always generate additional benefits in plant growth (Monsees et al., 2019), our study suggests that their presence, beyond yield, contributes to achieve a more uniform distribution of nutrients in the water, reducing the need for external adjustments. This aspect is fundamental to sustainability, as the reuse of nutrient-rich water significantly reduces greenhouse gas emissions (Monsees et al., 2019; Yildiz et al., 2017).

Likewise, the effect of plants on water quality originates a buffering effect on the pH evolution, and improved nitrate recirculation, as supported by previous studies such as that of Sabwa et al. (2022), who highlighted that an intermediate fish density maximizes plant biomass without compromising the system overall stability. Furthermore, research performed by Johnson et al. (2017) and Matysiak et al. (2023) found that the success of lettuce cultivation also depends on genotype. However, in our case, using just one lettuce variety, grown under controlled conditions, the differences found in growth between the systems with plants were due mainly to water quality.

Duarte et al. (2023) and Duarte & Cerozi (2024) have also addressed the contribution of micro- and macronutrients derived from fish and their evolution, emphasizing the role of total ammoniacal nitrogen (TAN) transformation as a key process in the evolution of the water quality. Our results support this hypothesis, as systems with biofilters and plants experienced an increased nitrification efficiency, which prevented critical nitrate accumulations and maintained stable calcium levels at the required level. The literature reviewed agrees that the addition of external nutrients can be useful for correcting specific deficiencies in water quality (Aslanidou et al., 2023; S. F. P. Duarte & Cerozi, 2024; Ru et al., 2017; Tsoumalakou et al., 2022), although a careful management of organic and inorganic inputs can avoid the addition of species such as Carbonated components (Bakhsh & Chopin, 2011; Harika, Ramakrishna Reddy, et al., 2024). In our study, the coupled systems maintained adequate water quality conditions, without the need for external interventions, reinforcing their potential as the achievement of low-impact, high-efficiency systems compatible with sustainable development.

On the other hand, various studies warn that the lack of water quality control can cause nutritional imbalances that directly could affect plant development (Maneepong, 2019; Márquez & García, 2022). In the present investigation, this was observed in the S3 system, where the absence of plants caused a significant accumulation of nitrates and Calcium in the last month of the growing cycle. This result indicates that the presence of vegetation not only improves nutrient absorption but also acts as an essential biological regulator to maintain the balance of the chemical species that allowed an improved water quality. Furthermore, it has been shown that even slight micronutrient deficiencies can interfere with essential physiological processes in plants, such as photosynthesis and gas exchange (Tsoumalakou et al., 2022). These results emphasize the need for constant monitoring and strategic adjustments to chemical elements, such as iron, potassium and calcium, as also was highlighted by (Cortez et al., 2009) when analyzing the use of aquaculture wastewater in hydroponics.

4.7 Preliminary conclusions

This study analyzed the interactions between water quality dynamics and biomass production of *Oncorhynchus mykiss* and *Lactuca sativa* in a coupled Recirculating Aquaponic System (RAS) over eight months, comprising one trout production cycle and six successive lettuce growing cycles. The results found revealed a direct relationship between the evolution of pH, alkalinity, calcium, and nitrogen species (NO_2^- -N and NO_3^- -N) and the growth performance of both organisms. A novel contribution of this work is the transient analysis of the dynamic balance between NO_2^- -N and NO_3^- -N, and its correlation with fish growth in systems with and without lettuce cultivation.

The presence of lettuce played a critical role in maintaining the quality of water, as the hybrid systems coupled with plants, originated an improvement in the chemical stability, particularly in buffering the nitrogen fluctuations. Coupled lettuce cultivation contributed to a more uniform distribution and a lower accumulation of nitrogenous compounds, supporting healthier conditions that favored the fish growth rate.

Notably, trout reared in systems with lettuce exhibited an increment of 14.7% in the final biomass compared to the uncoupled system, highlighting the ecological and productive benefits of an integrated plant production. Additionally, the inclusion of lettuce added economic value to the system while reinforcing the functional role of biofilters in sustaining microbial activity and nitrogen cycling.

These findings underscore the importance of holistic system design in aquaponics, where plant integration is not only a complementary component but a fundamental driver of water quality regulation that allowed and improved fish performance.

CHAPTER 5

LIFE CYCLE ASSESSMENT FOR SUSTAINABLE INTEGRATED TROUT – LETTUCE SYSTEM

5.1 Abstract

The present study assessed the environmental performance of an integrated aquaponic system combining trout (*Oncorhynchus mykiss*) and lettuce (*Lactuca sativa*) production, under controlled greenhouse conditions in the semi-arid region of Coquimbo in the north of Chile. A gate-to-gate Life Cycle Assessment (LCA) was applied to evaluate environmental indicators, including Water Use Efficiency (WUE), Specific Energy Consumption (SEC), Cumulative Energy Demand (CED), and Global Warming Potential (GWP), over a nine-month production cycle. Results indicate that lettuce exhibited consistently higher resource efficiency, with WUE values between 61 and 101 kg/m³ and SEC ranging from 145 to 255 MJ/kg, in agreement with reported data. Conversely, trout production showed greater variability, with WUE values in the interval between 18 and 36 kg/m³ and SEC ranging from 490 to 890 MJ/kg. Water intensity (WI) for trout increased throughout the cycle from 1.85 to 4.1 m³/kg. In contrast, lettuce WI remained low (1.0–1.3 m³/kg), as a result of stable evaporative and recirculatory conditions. GWP values ranged from 6.2–12.4 kg CO₂-eq/kg for lettuce and 29.5–58.7 kg CO₂-eq/kg for trout, largely due to electricity consumption and feed transport. These results confirm that the vegetal component of the aquaponic system is more environmentally efficient in terms of both water and energy use, while the fish component imposes higher environmental burdens like other intensive aquaculture systems. This study provides novel, empirical evidence supporting the environmental viability of coupled aquaponic systems to provide healthy foods, and identifies critical hotspots for optimization, particularly in solid waste management, energy demand, and nutrient recovery for the combined lettuce and trout production in a semi-arid region.

5.2 Literature review

Sustainability in food production represents a multifaceted challenge that requires balancing environmental care, economic viability, and social equity. Ensuring the ability of future generations to meet their food needs depends not only on the increment in production, but at the same time, on implementing ways that minimize ecological degradation and promote long-term resilience by growing high quality foods.

The achievement of this balance involves integrating traditional practices with innovative technologies that enhance productivity while reducing the pressure on natural resources. In agriculture, practices such as crop diversification and hydroponics contribute to improved soil health and biodiversity, helping to reduce the incidence of pests and diseases. Techniques like crop rotation are key to maintaining soil fertility while promoting ecological stability (Sadia et al., 2025). Water management strategies, including drip irrigation and rainwater harvesting, play a crucial role in regions facing water stress, making irrigation more efficient and sustainable (Sharma et al., 2024). At the same time, the incorporation of innovative systems such as hydroponics, aquaponics, and vertical farming allows for year-round food production using less land and water (Audu Baba and Adamu, 2021; Certini and Scalenghe, 2023; Khattak et al., 2025). These approaches are especially relevant in urban and arid areas in the actual global change scenery.

In the context of aquaculture and aquaponics, numerous studies have identified fish feed as the primary input responsible for the most substantial environmental impacts, primarily due to its role in providing essential proteins and nutrients for carnivorous fish species such as trout (D'Orbcastel et al., 2009; Guan et al., 2025; Santos et al., 2015). In the field of aquaculture, sustainability is closely tied to reducing environmental impact through improved waste management and pollution control (Blanchard et al., 2017; Ytrestøyl et al., 2015). Emerging technologies aim to minimize nutrient discharge into natural ecosystems, while increasing the circularity of inputs. Aquaponics exemplifies this approach by integrating fish and plant cultivation, recycling nutrients, and reducing water use, thus contributing to a circular

food economy. Governance and policy support are also fundamental in ensuring that growth in food production does not compromise biodiversity or climate targets. Regulatory frameworks and inclusive policies enable responsible scaling of sustainable practices. Despite the progress achieved, significant barriers remain, including the integrated analysis of the effects of climate variability, resource limitations, and socio-economic disparities (Andika et al., 2025; Halpern et al., 2024; Polman, 2025; Rabbi and Amin, 2024).

Aquaponics represent a promising integrated and circular approach to sustainable food production by combining aquaculture and hydroponics in a mutually beneficial system (Ibrahim et al., 2023; Iqbal et al., 2025; Lily, 2024). This method addresses the increasing global demand for food while enhancing resource efficiency through the recirculation of water and nutrients (Obirikorang et al., 2021). In aquaponic systems, fish waste is biologically converted into nutrients for plant growth, eliminating the need for synthetic fertilizers and reducing environmental impact (Goddek et al., 2019). This closed-loop configuration not only minimizes water use—by up to 90% compared to conventional agriculture—but also fosters biodiversity through the integration of aquatic and plant life, creating more resilient ecosystems (Behr et al., 2025; Zoli et al., 2024).

Beyond environmental benefits, aquaponics offers opportunities for economic diversification and localized food production. While the initial setup may involve high costs, especially for larger-scale or technologically advanced systems, small and medium-scale operations have demonstrated financial viability, particularly in peri-urban settings where proximity to markets enhances profitability (Okomoda et al., 2023). By producing both healthy animal protein and vegetables within a single system, aquaponics can provide multiple income streams for producers, increasing resilience to the market fluctuations (Henriksson et al., 2025).

However, challenges still remain. The technical complexity of balancing water quality, nutrient levels, and species needs requires both expertise and reliable monitoring systems. Energy demand, particularly for water circulation and temperature control, can also impact the operating costs and sustainability,

especially in regions with limited access to renewable energy sources. Moreover, the lack of standardized regulatory frameworks and limited training opportunities pose barriers to wider adoption. To unlock the full potential of aquaponics in global food systems, it is essential to address these obstacles through supportive policies, accessible education, and context-specific technological innovation (Edwards et al., 2024; Makinde and Arilekolasi, 2025; Wolfaardt et al., 2025).

The environmental impact of aquaponics remains insufficiently understood, revealing a critical gap in the current body of research in this area. Although aquaponics is widely promoted as a sustainable method for food production, there is a lack of comprehensive data to support its environmental performance, which complicates evidence-based policymaking and hinders broader implementations. One of the key limitations lies in the narrow focus on a limited selection of aquatic and plant species, which restricts the generalizability of many findings (Behr et al., 2025; Bhakar et al., 2021; Liu et al., 2016). Additionally, methodological inconsistencies, particularly in the definition of system boundaries and scope, have led to varied and sometimes conflicting conclusions about the actual environmental impact of aquaponic operations. Life Cycle Assessments (LCA), which offer a holistic view on the efficient use of resources and the emissions generated, have only been sporadically applied to some specific aquaponic systems, limiting the ability to compare them accurately with other food production methods (Greenfeld et al., 2021; Kamareddine and Maraqa, 2021). Furthermore, crucial aspects such as electricity consumption and the role of fish feed in overall sustainability have not been adequately quantified. The integration of aquaponics into urban contexts presents additional challenges, and therefore more research is needed to understand how these systems interact with urban metabolic flows and infrastructure (Proksch and Ianchenko, 2023). Despite these gaps, some researchers suggest that the inherent benefits of aquaponics, such as the drastic reduction in water usage and the minimized need for chemical fertilizers, may outweigh the current limitations in our understanding, reinforcing the importance of further research rather than delaying practical applications (Kamareddine and Maraqa, 2021)

The objective of this research is to evaluate the environmental sustainability of a trout–lettuce aquaponic system by estimating its energy and water consumption through a Life Cycle Assessment (LCA) approach. The study is grounded in experimental data collected over a nine-month period, which includes eight consecutive lettuce crop cycles along one full trout production cycle, under real operational conditions at a pilot scale. Unlike previous research that often relies on simulations or theoretical assumptions, this study introduces a novel practical-theoretical methodological approach by working with measured data across different system configurations (Henriksson et al., 2025; Lobanov et al., 2025). The analysis incorporates a comprehensive inventory of the key inputs and outputs, including smolt transportation, commercial feed, electricity use, water consumption, seedling production, substrate materials, and infrastructure components, alongside outputs such as harvested trout and lettuce biomass, residual water, energy budget and emission metrics. This integrated experimental design and scenario-based comparison provide valuable insights into the environmental trade-offs and potential improvements for aquaponic systems in water- and energy-constrained settings.

5.3 Goal of the study

The objective of this study is to quantitatively assess the environmental performance of a bio-integrated aquaponic system that simultaneously cultivates rainbow trout (*Oncorhynchus mykiss*) and lettuce (*Lactuca sativa*) under controlled greenhouse conditions in the semi-arid environment of northern Chile. By applying a gate-to-gate Life Cycle Assessment (LCA) approach, the study aims to determine the resource efficiency and environmental impacts associated with both the aquaculture and hydroponic components of the system.

The analysis focuses on key indicators, including Water Use Efficiency (WUE), Specific Energy Consumption (SEC), Cumulative Energy Demand (CED), and Global Warming Potential (GWP), in order to establish a comparative understanding of how each productive component contributes to the overall environmental footprint. In addition, the study seeks to identify critical hotspots—such as electricity consumption, feed transport, and waste management—that significantly influence

system sustainability. Ultimately, the research aims to generate empirical evidence supporting the environmental feasibility of integrated aquaponic systems as a sustainable alternative to conventional agriculture and aquaculture, contributing to the design of resource-efficient, low-impact food production systems suitable for arid and semi-arid regions.

5.4 Methodology

The methodology used to implement the life cycle assessment (LCA) was based on an experimental approach, using real data, as described below.

5.4.1 Experimental design

The Recirculating Aquaponic System (RAS) evaluated was located at the Pan de Azucar Experimental Parcel, part of the Agricultural Research Institute (INIA, Chile) in Coquimbo, Chile (30°4'14.73"S, 71°14'32.56"W). The system was composed of two identical modules (S1 and S2), each including an aquaculture component dedicated to Rainbow Trout cultivation (*Oncorhynchus mykiss*), and a horticulture component for Lettuce production (*Lactuca sativa*). Both components were housed within a greenhouse structure, specifically designed for hydroponic production. The design and dimensions of both greenhouse and hydroponic beds are shown in Figure 52.

The two modules were operated in tandem (S1 and S2), where the effluent of each one was directed to a settler tank for the decantation of the suspended solids. The treated water then flowed through two biofilters, with part of the effluent being routed to a water accumulator operating as a Sump. Finally, the physically and biologically treated water was directed into two hydroponic lettuce beds operating in Deep Water Culture technique (DWC). The effluent from these was recirculated back into the system.

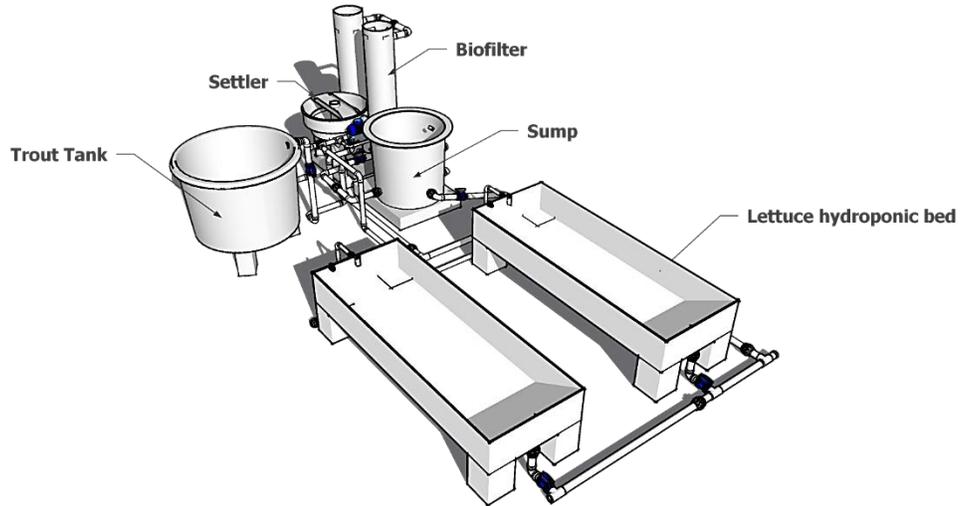


Figure 52. Layout of one of the aquaponic system modules (S1 or S2), where Trout effluent is sent to a settler tank, and from there it is treated in biofilter before passing to the lettuce hydroponic beds. (Module built with grant ANID IT 20I0066).

Water circulation in each module was maintained using a 1 HP pool pump (Vulcano BAP model), while aeration was provided by a 1 HP blower pump. These devices constituted the only electrical equipment required to operate each system for one crop cycle. This equipment also provided pumping and aeration for the hydroponic beds in each system. Recirculated water volume and culture units used in both systems are detailed in Table 11.

Table 11. Culture units and water volume of components of one module, S1 or S2.

Unit	Individual modular volume	Units per module	Total aquaponic volume per module
Trout Tank	1 m ³	1	1 m ³
Lettuce hydroponic bed (DWC)	0.5 m ³	2	1 m ³
Submerged biofilter	1.2 m ³	2	2.4 m ³
Settler	0.5 m ³	1	0.5 m ³
Sump	1.5 m ³	1	1.5 m ³
Total Water Volume per system			6.4 m³

5.4.2 Sizing and design of the Recirculating Aquaponic System (RAS)

The sizing and design of RAS components were carried out using mass balance procedures. Mass balance is a fundamental approach to identify and quantify the inputs, outputs and internal transformations within a system (Hutchinson, 2004). This approach allows tracking the movement and transformation of species into the different components of the system. The general equation used for this analysis assumes stationary conditions,

$$(Q_m \times C_m) + (Q_r \times C_r) + P = (Q_m \times C_{\text{tank}}) + (Q_r \times C_{\text{tank}}) \quad (26)$$

where Q_m is the inlet flow rate (L/min), C_m is inlet concentration (mg/L), Q_r the new flow rate, (L/min), C_r concentration of the new flow (mg/L), P corresponds to production, (mg/min) and EC_{pond} the concentration in the pond (mg/L) (Wolters et al., 2009).

Each calculation included a set of specific variables that were considered for the different treatment processes. A detailed mass balance was performed for Nitrate (NO_3^{-2}) and Total Ammoniacal Nitrogen ($\text{NH}_3 + \text{NH}_4^+$). The Dissolved Oxygen (DO) levels required to maintain adequate fish growth and the required flow rate (Q) for the entire system and its water treatment units were calculated by suitable conversions and pertinent assumptions.

5.4.3 Infrastructure and equipment

Weight and quantity of each culture unit and equipment installed in the experimental system were calculated. Through a literature search, CO_2 emission values during the LCA were obtained (Table 12), which were subsequently amortized monthly (Wernet et al., 2016).

Table 12. Quantity estimation and CO₂ emissions of the main structural components used in both aquaponic modules, S1 and S2.

Unit	Total units	Material	Estimated weight (kg)	Emission (kg CO ₂ e/kg)	Total Emission (kg CO ₂ eq)
Hydroponic bed	4	Wood	240	0.72	172.8
Fish tank	2	Fiberglass	300	1.05	315
Air blower 1HP	2	Steel	50	3	150
Water pump 1HP	2	Steel	50	3	150
Metal structure	1	Galvanized steel	330	2.95	973.5
Greenhouse covering	1	Polyethylene	13.2	2.7	35.64

Detailed information on the experimental aquaculture and horticultural components in both systems, S1 and S2, is provided below.

5.4.4 Experimental Aquaculture component

A total of 130 Rainbow trout (*Oncorhynchus mykiss*), each weighing approximately 60 g, was transported by truck, with a diesel engine, from the Río Blanco fish farm in the Valparaíso Region to the commune of Coquimbo, located 456 km away in the Coquimbo Region, Chile. This facility was the closest source of fry to the experimental site. Upon arrival, the fish were stocked into tanks at a final density of 30 kg/m³. Over a nine-month culture period, this density was maintained, ensuring a stable number of trout throughout all developmental stages. Feeding was carried out with a ration equivalent to 3% of the total biomass, divided into two daily intakes. BIOMAR® feed, specifically formulated for the growth stages of the trout, was used, and acquired twice during the whole experiment, being transported by truck from the city of Puerto Montt, Chile, to the experiment site, covering a transport distance of 1,500 km.

Following the guidelines of Rakocy et al. (2004), which state that 1 m² of vegetables can be supported per 60 g of fish feed, the fish were introduced first. After administering 60 g of feed, plants were added to the system. Vegetables were planted at a density of 25 plants per m². No chemicals or nutrient solutions were added at any time during cultivation, and no pests or diseases were reported in the fish or plant systems. To evaluate the growth in weight of the fish, they were sampled monthly and the amount of feed to be fed was established.

5.4.5 Horticultural component

Lettuces grown during all the cycles evaluated corresponded to the Quenty variety and were obtained from a local greenhouse located 5 km from the area where the culture was carried out. 400 seedlings were acquired for each cycle, each cycle of lettuce lasted between 35 and 45 days, and 360 of them were transplanted for all four hydroponic beds. In the plant component, leaf and root growth was assessed weekly in each hydroponic bed, with a representative sampling of 60% of the total number of plants. At the time of harvest, the totality of each bed was analyzed to determine the final biomass produced.

Figure 53 illustrates the growing of trout and lettuce crops in terms of total biomass, during the experiments, from October 2023 to May 2024, in subsystems S1 and S2. The vegetable biomass progressively increased in both systems (S1 and S2), with a 12.5% higher growing rate for S2, from February 2024 on. Additionally, this figure depicts that the highest biomass production, 95 kg for S1 and 120 kg S2, was achieved in May 2024, mainly due to a reduction in the trout biomass in December 2023.

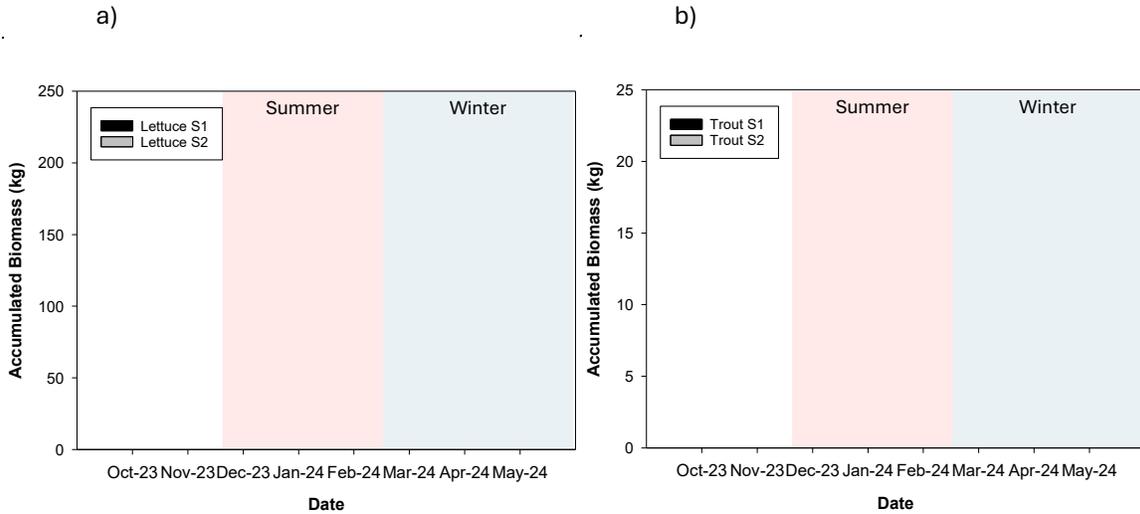


Figure 53. Evolution of accumulated lettuce biomass (a) and trout (b) in subsystems S1 and S2, from October 2023 to May 2024, in red box warmer, and in blue box cooler season.

As for the fish component, the increase in biomass of the fish in each subsystem was monitored monthly, using periodic sampling to estimate the necessary feed rations. Finally, in the harvesting stage, the entire population present in each subsystem was counted to obtain the final production values (Table 13).

Even though at the end of the experiment, May 2024, the total trout biomass of both systems (S1 and S2), was identical, the evolution of the growing trend was different, with trout in S1 exhibiting a lower growing rate during the first seven months. This can be explained because in this system the nitrifying biofilter did not mature at the same rate.

Table 13. Stocking density for the fish and lettuce RAS module.

Unit	Total quantity	Density	Stock	Species number
Trout Tank	2	30 k/m ³	1	116
Lettuce hydroponic bed	4	25 plants/m ²	2	60*4*6

5.4.6 Determination of emissions - LCA model

Based on the design of the experimental aquaponics system and its structural components, the quantity of materials used for each of the units comprising it was estimated. This information was used as the basis for subsequent emissions calculations within the framework of the Life Cycle Assessment (LCA) as depicted in Figure 54.

An extended Gate-to-Gate model was used for this study, covering all inputs and outputs from the start of monthly production (seedling planting and smolt entry) to the final product (fish and vegetable biomass), excluding stages prior to input production (cradle) and distribution or subsequent use of the final product. This approach was selected due to the availability of accurate data on direct inputs, energy consumption, water and monthly production yield, which allows for exact traceability within the operating unit. However, certain infrastructure and transport elements were incorporated, making this model an intermediate approach between Gate-to-Gate and partial Cradle-to-Gate, adapted to the actual conditions of the trials.

5.4.7 Weighted Emission Factor (WEF)

To refine the estimation of emissions derived from Energy Use Efficiency (EUE), a Weighted Emission Factor (WEF) was calculated based on the proportional contribution of each energy source in the Chilean electricity mix for 2023. This factor integrates the emissions intensity (in kg CO₂ e/kWh) of both renewable and non-renewable technologies, assigning greater weight to the most represented sources. For instance, hydro, solar, and wind were considered to have negligible or zero direct emissions, while fossil-based sources like coal, natural gas, and oil retained their respective high emission factors.

$$WEF = \sum_{i=1}^n (p_i \cdot f_i) \quad (26)$$

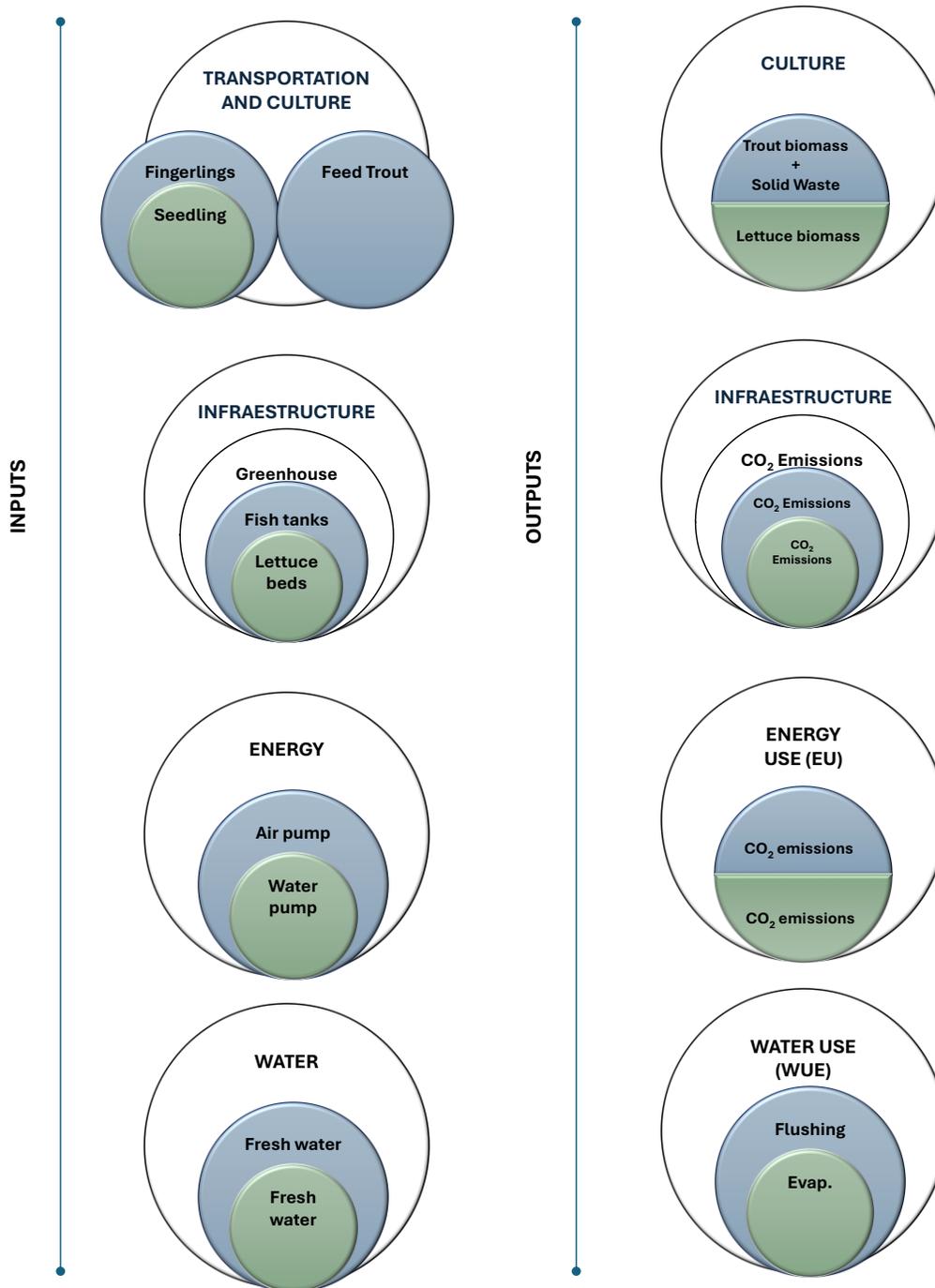


Figure 54. Inputs and outputs used for LCA, in components culture, transport, infrastructure, energy and water of experimental aquaponic system.

where p_i is the proportion (%) of the total electricity mix from source i , f_i is the emission factor of source i (in $\text{kgCO}_2 \text{ e/kWh}$), and n corresponds to the number of energy sources considered. The WEF value for water pumps and air blowers obtained for the national energy mix was $0.228 \text{ kgCO}_2 \text{ e/kWh}$, which was then applied uniformly across all electricity-consuming components of the aquaponic system, and is detailed in the Table 14. The approach used provided a representative estimate of the real emissions associated with the national grid rather than relying on standard or default emission values. This context was considered when calculating the carbon footprint of the RAS system, considering the specific energy consumption of each equipment and its monthly impact. To determine the energy consumption of the equipment, the energy equation (Eq. 28) was used, where E represents the energy consumption in kilowatt-hours (kWh), P is the power of the equipment in kilowatts (kW), and t corresponds to the operating time in hours.

$$E = P \cdot t \tag{28}$$

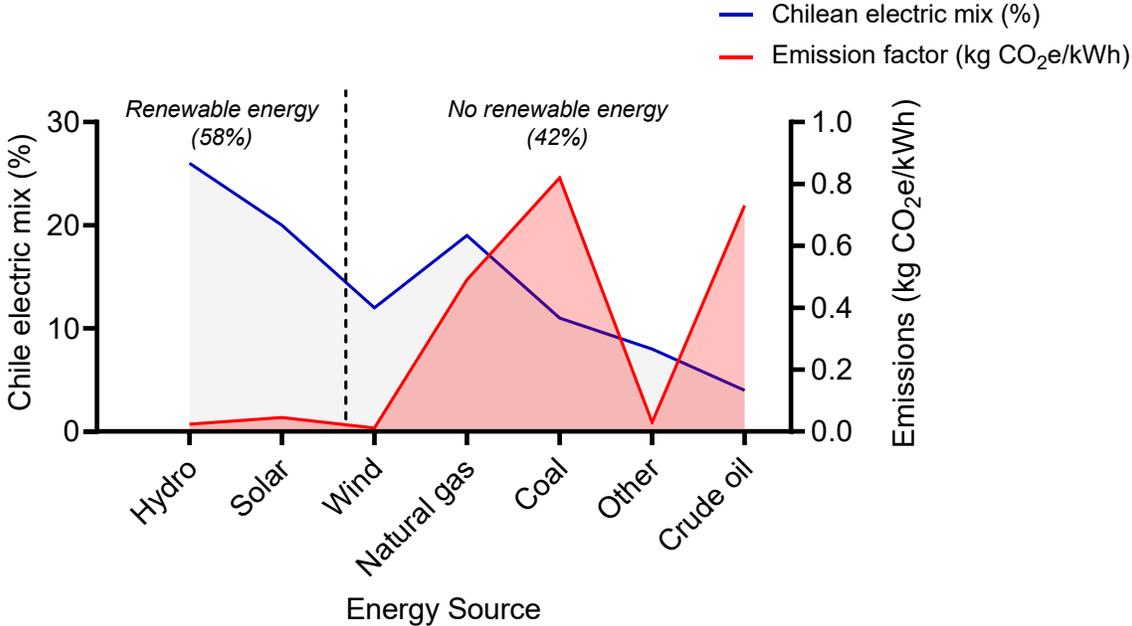


Figure 55. Energy mix data published by the Chilean National Electricity Coordinator (CEN), for the year 2023 (left axis), and Weighted Emission Factor (WEF) calculated (right axis).

The energy cost was calculated by multiplying the total energy consumption by the unit cost of the electricity tariff, given as 0.117 USD/kWh, for the nine months of continuous operation (Eq. 29).

$$E_{cycle, total} = E_{cycle, pump} + E_{cycle, blower} \quad (29)$$

5.4.8 Energy Use Efficiency (EUE)

Recirculating Aquaponic System (RAS) operated continuously throughout the nine-month trial, providing controlled conditions for cultivating both *Oncorhynchus mykiss* (Rainbow trout) and *Lactuca sativa* (Lettuce). The system was powered from the domestic electricity grid, with two 1-HP water pumps and two 1-HP air blowers running 24/7. Monthly electricity use was computed from installed power and runtime (Eq. 30).

$$kWh_{month} = (\sum P_{devices}(kW)) \times 24 \times d \quad (30)$$

where $P_{devices}$ represents the nominal power of each electrical component (kW), and d was the number of days in the month. Energy Use Efficiency (EUE) related productive output to the electricity consumed in the same period by the expression,

$$EUE = \frac{Biomass\ produced\ (kg) \times 1000}{Electricity\ (kWh)} [gkWh^{-1}] \quad (31)$$

To calculate the emissions associated with the electricity consumption, the emission factor corresponding to the national energy matrix of Chile for 2023 was used. According to data from the National Electricity Coordinator (2023), this matrix has a mixed composition, with 58% coming from renewable sources (including solar, wind, hydro and biomass), while the remaining 42% comes from non-renewable sources, mainly coal, natural gas and oil. Despite progress in decarbonization, emissions intensity remains considerable high due to the integration of thermoelectric technologies with high CO₂ equivalent emission factors (kgCO₂e/kWh).

$$EF_{electricity} = 0.329 \text{ kg } [CO_{2-eq}/kWh] \quad (32)$$

Therefore, the emissions derived from energy consumption were computed as:

$$CO_{2 \text{ electricity}} = Electricity \text{ consumed} \cdot EF_{electricity} \text{ (kg } CO_{2-eq}) \quad (33)$$

5.4.9 GWP (Global Warming Potential)

The estimation of Electricity-Related Emissions allowed the calculation of the GWP (Global Warming Potential) associated with electrical use, and by dividing by the biomass output, the GWP-specific emission intensity was derived as,

$$GWP_{specific} = \frac{CO_{2 \text{ electricity}}}{kg \text{ biomass}} \left(\frac{kg \text{ } CO_{2-eq}}{kg} \right) \quad (34)$$

5.4.10 Water consumption (WUE)

Water consumption in the RAS was continuously monitored throughout the nine-month operational period. This monitoring considered both the internal water dynamics of the system and external factors contributing to water loss. Two dominant pathways were quantified: (i) evapotranspiration (ET), driven by the greenhouse microclimate, and (ii) routine flushing of settling units to remove solids and maintain the water quality. ET was estimated through a semi-empirical mass-balance model adapted to controlled environment aquaponics and measured by the greenhouse temperature and the relative humidity recorded under the polyethylene cladding.

Following the guideline of Allen et al. (1998) and modifying coefficients to leafy crops in semi-closed conditions, the monthly ET was estimated as:

$$ET_{monthly} = k \cdot (T_m + 5) \cdot S \cdot d \quad (35)$$

where T_m was the monthly average air temperature inside the greenhouse ($^{\circ}\text{C}$), S was the evaporative surface (12 m^2 ; four DWC beds of $3 \times 1 \text{ m}$), d was the number of days in the month, and k was an empirical coefficient, between 0.1 and 0.2), for broad-leaf canopies under high-radiation conditions; were the value $k=0.2$ was used for open beds with *Lactuca sativa*.

The values calculated of ET showed marked seasonality in the south hemisphere, reaching a peak value in warmer months (e.g., February, $\sim 7.2 \text{ L m}^{-2} \text{ day}^{-1}$) and declining in cooler months (e.g., May, $\sim 3.1 \text{ L m}^{-2} \text{ day}^{-1}$). The make-up water added to compensate for ET and the volumes purged during flushing were logged monthly, yielding the total system-level water demand reported in Figure 56.

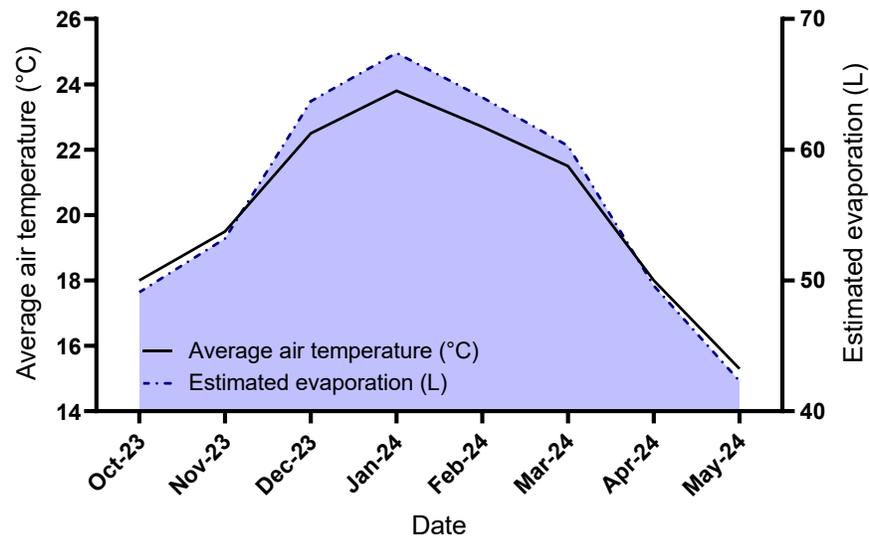


Figure 56. Average temperature inside the greenhouse and estimation of water losses due to evapotranspiration.

From this data, the water use efficiency (WUE) was determined as the ratio between the total volume of water consumed (in Liters) and the combined biomass production (in kilograms) of *Lactuca sativa* and *Oncorhynchus mykiss* for each month, as shown by Equation 36 (Mohammedp et al., 2016),

$$WUE = \frac{\text{Total volume of water consumed (L)}}{\text{Total biomass produced (k)}} \quad (36)$$

The scientific literature related to hydroponics usually reports the reciprocal value of equation 11, therefore, the present work also provides this information in g L^{-1} where helpful (i.e., biomass-based $\text{WUE} = (\text{biomass (kg)} \times 1000)/\text{water (L)}$). Additionally, normalizing the water use by the cultivated area (L m^{-2}) is frequently reported in the literature to facilitate benchmarking against conventional hydroponic and aquaculture systems.

5.4.11 Life Cycle Assessment impact

The elementary flows and emission sources identified throughout the system were converted into their respective impact contributions using standardized characterization factors. The Global Warming Potential (GWP) was calculated using the IPCC AR5 characterization factor of $0.329 \text{ kgCO}_2\text{e/kWh}$, consistent with the Chilean electricity grid emission factor for 2023. The Acidification Potential (AP) and Eutrophication Potential (EP) were estimated by applying midpoint characterization factors from the CML-IA baseline method (Van Oers et al., 2016), using sulfur dioxide and phosphate equivalents per process respectively. For Cumulative Energy Demand (CED), electricity consumption was converted to megajoules using the conversion factor $1 \text{ kWh} = 3.6 \text{ MJ}$, following the energy intensity coefficients implemented in Simapro v9.2. The impact categories Water Depletion (WD) and Freshwater Ecotoxicity (ECO) were approximated based on default midpoint factors from ILCD 2011 Midpoint+ v1.10 (European Commission, 2012), adjusted to regionalized water consumption data and effluent assumptions. The calculations assumed a gate-to-gate system boundary, with cradle-to-gate emissions included only for upstream inputs such as fish feed, seedlings, and transport.

5.5 Results

5.5.1 Environmental Energy assessment

The design and equipment currently in operation were considered in the calculation procedure to estimate energy consumption. The energy analysis was based on the

actual operating conditions of the coupled fish-vegetable system, during the nine-month growth cycle for trout, up to a final weight of 500 g, in which six complete lettuce cycles were successfully accomplished.

5.5.2 Energy consumption

Considering the operation of the equipment used during the nine months of production, the total energy cost amounted to 8,593.92 USD.

The Energy Use Efficiency (EUE, kg/kWh) of the aquaponic system increased progressively over the nine-month operational period, ranging from approximately 0.09 kg/kWh in October 2023 to values above 0.20 kg/kWh in May 2024. Lettuce subsystems (S1 and S2) provided a consistent and stable contribution throughout the experiment, sustaining baseline efficiency during the early stages of production. In contrast, trout subsystems (S1 and S2) showed a more variable performance, with a marked increment from February 2024 onwards, coinciding with the growth acceleration during the fattening phase.

The increase in EUE demonstrates the advantage of integrating fish and plant production within a single coupled production system. While lettuces contribute early and consistently to the biomass-to-energy ratio, trout growth becomes critical in the later stages, improving the overall energy balance. This synergy highlights how coupling species with different growth dynamics enhances the long-term efficiency of the electricity use, as depicted in Figure 57.

When contextualized with the energy matrix of Chile, where 58% of electricity in 2023 originated from renewable sources and 42% from fossil-based thermoelectric plants, the results gain further significance. By achieving higher EUE values in later months, the system reduced the relative carbon intensity (kgCO₂ e/kWh) of production, as more biomass is obtained per unit of energy consumed. The integrated approach not only diversifies production but also dilutes the environmental burden of the use

of electricity, contributing to lower per-unit emissions and reinforcing the sustainability potential of aquaponics in semi-arid regions.

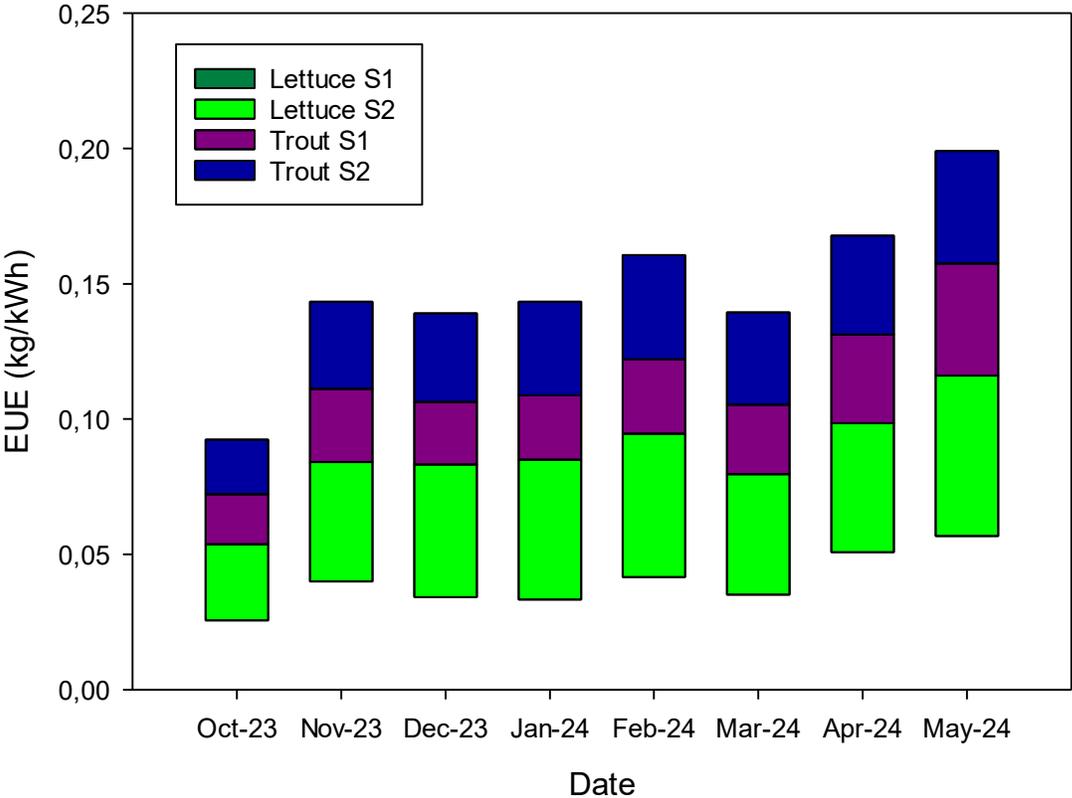


Figure 57. Monthly Energy Use Efficiency (EUE, kg/kWh) of lettuce and trout in systems S1 and S2.

5.5.3 Water consumption

At the end of each vegetable cultivation cycle, evapotranspiration losses were calculated, averaging 10% of the total volume of water in the system. These losses were replenished with well water, amounting to 200 L per cycle and a total of 1.6 m³ over the evaluation period.

During the first six months of the production cycle, Water Use Efficiency (WUE) values remained relatively stable, ranging between 10.1 and 13.8 kg/L, with an

approximate average value of 11.9 ± 1.4 kg/L. This stability reflects a balance between the early growth of lettuce and the initial development of trout, with no sudden increases in the total biomass. From April 2024 onwards, the trend changed significantly, with an increment close to 40% compared to the previous month, reaching a value of 17.5 kg/L, and culminating in May with a maximum of 25.3 kg/L, representing a cumulative increment approximately equal to 110% compared to the average value achieved during the initial months.

The contribution of lettuce in systems S1 and S2 remained relatively constant throughout the period, with values fluctuating between 0.8 and 2.1 kg/L, equivalent to less than 15% of the total monthly WUE. This behavior confirms its stabilizing role in the system: an early and sustained contribution of short-cycle biomass, with low inter-monthly variability. In contrast, trout shows a completely different dynamic. Trout S1 contributed steadily to the first few months (between 4.8 and 6.2 kg/L) but increased its share in April (7.5 kg/L) and markedly in May (14.1 kg/L). The values of WUE for trout in the system S2 were even more determining: starting with values around 4.5 kg/L in the first months, it reached 8.0 kg/L in April and a maximum of 11.9 kg/L in May, accounting for almost half of the total WUE in the last month of cultivation. This difference in input patterns confirms that, while lettuce ensured a constant basis of water efficiency, it was trout growing, particularly in the S2 subsystem, that originated a significant increment in the final phase of the cycle, linked to the accelerated biomass growth during fattening, as indicated in Figure 58.

Figure 59 illustrates the generation of CO₂ during the production of the coupled lettuce-trout cycle. The specific GHG intensity ($\text{kg CO}_2\text{e}\cdot\text{kg}^{-1}$) exhibited seasonal dynamics for two main reasons: (i) a continuous energy load, which penalizes the months of low production, and (ii) intermittent background flows, such as bi-monthly feed transport, monthly seedling transport, and the single transfer of fry at the beginning of the cycle. These combined effects explained the reasons why the value was higher in the first months, when total production was still very low and start-up activities were concentrated, including smolt transport and the first feeding.

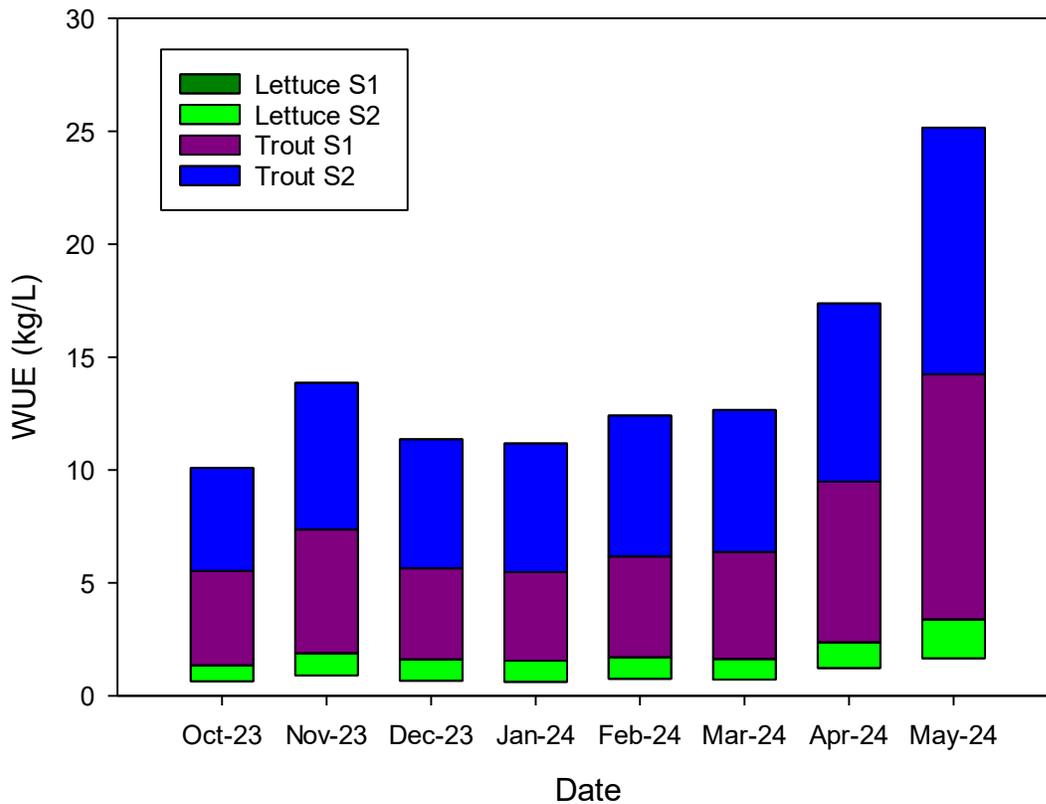


Figure 58. History of the monthly Water Use Efficiency (WUE, kg/m³), for lettuce and trout hybrid aquaponic systems S1 and S2.

The improvement on WUE between January and February was due to an increase in monthly biomass, which reduced the electricity and infrastructure amortized per kg, and the relatively lower share of transport. The upturn between March and May coincided with new feed deliveries and a higher relative weight of trout in the allocation of loads, which increased the specific contribution of that chain, including the long-distance logistics.

A critical aspect was the allocation of loads between co-products. Here, water was distributed in a ratio of 90/10 (plants/fish) and energy 50/50, while electricity and infrastructure dominate the footprint. This criterion is justified for a coupled system, but it conditions the specific footprint per species: if S2 produced more lettuce, its kg

of product originated less CO₂ eq for the same energy, while trout inherits proportionally more load when its share in the mix grows.

Operationally, the hot spots remain electricity (main contributor; mitigable with demand control, hydraulic pulses/efficient aeration or NCRE contract), food transport (optimizable by routes/frequency and nearest supplier) and, to a lesser extent, seedlings (local but monthly). Reducing hours/HP or improving the productive plant factor (= more kg for the same power) linearly depressed GHG intensity; similarly, decarbonization of the mix or dedicated PV would have the greatest absolute impact on the monthly footprint.

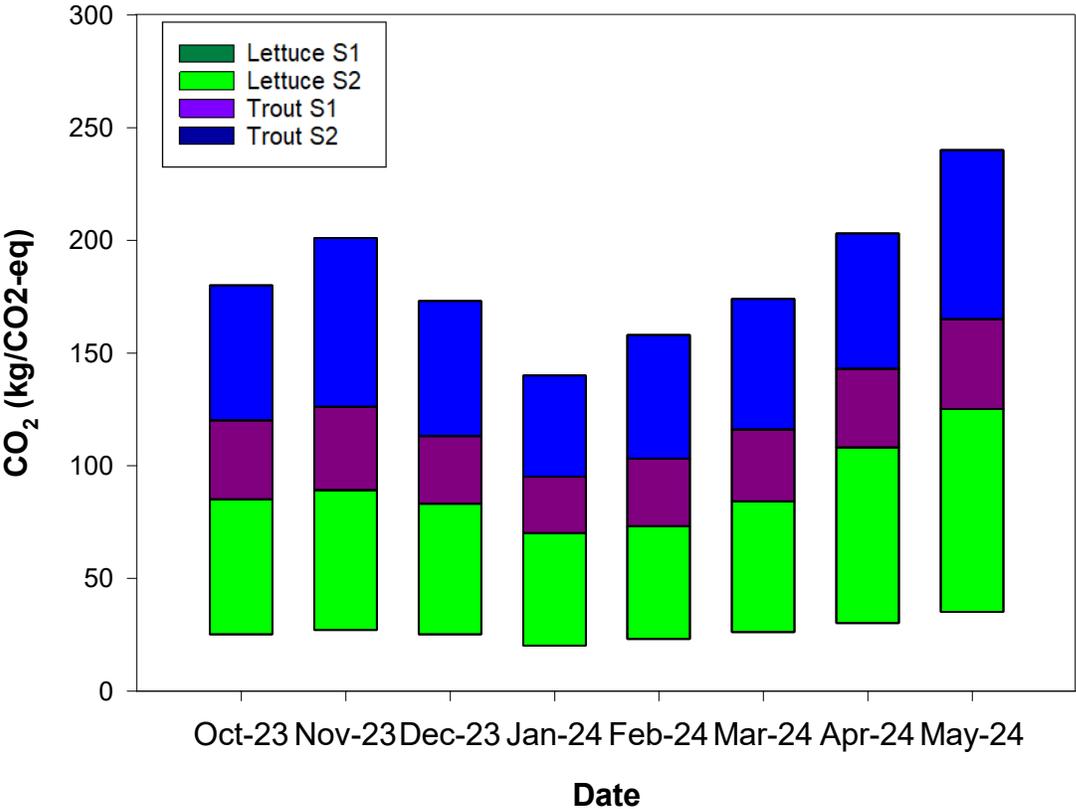


Figure 59. CO₂ generation during the production cycle of lettuce and trout in the coupled system.

In order to be able to compare optimal the CO₂ production, the weighted value (%) was calculated, which is the percentage composition (%) of the emissions per item and category, and corresponds to a normalised value of the emission of the item with respect to the total shown.

$$\text{Weighted value (\%)} = 100 \cdot \frac{E_i}{E_{total}} \quad (37)$$

where E_i is the emission of item i in kg CO₂ - eq, and E_{total} is the sum of the emissions of all submitted items.

The results indicated that infrastructure accounted for approximately 47% of the total CO₂e emissions. Within this category, the three main components of similar magnitude were: hydroponic beds (12.1%), cultivation tanks (11.9%) and biofiltration systems (11.8%); while the greenhouse structure added another 8.4%. This pattern is consistent with pilot-scale systems, where the incorporated footprint of structural materials dominates the initial cycle loads.

Transport contributed around 29% of the total emissions of CO₂, with significant contributions from input transport (9.0%) and food transport (8.5%), reflecting long and frequent logistics chains, especially for key inputs. In the input production of CO₂, the combined contribution reached 14.9%, led by fertiliser production (7.3%), while seedling/seed production accounted for approximately 2%.

5.5.4 Environmental Impact Assessment: Carbon and Energy Footprint

A joint assessment of the energy productivity (kg/MJ) and the Specific Energy Consumption (SEC; MJ/kg) of lettuce and trout between October 2023 and May 2024 reveals contrasting trends which require further analysis. Although the two indicators were inversely related, they allow us to elucidate the energy performance of the aquaponic system at species and temporal levels. First, lettuce consistently

exhibited higher energy productivity than trout, reaching values above 40,000 kg/MJ by the end of the period, whereas trout did not exceed 15,000 kg/MJ (Fig. 60).

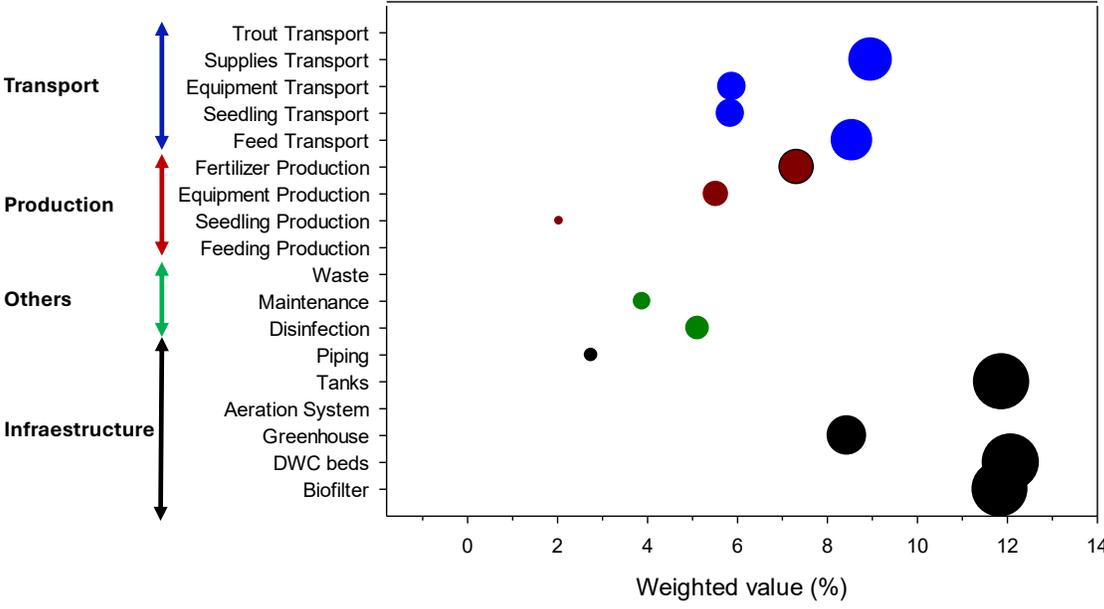


Figure 60. Accountability of CO₂ emissions with the weighed value for each item.

This difference indicates that, from an energy perspective, vegetable cultivation has a higher conversion rate of electrical energy into biomass. This was due to its lower metabolic requirements, the absence of thermal demand and its direct response to photo-period and dissolved nutrients from the fish. In contrast, as a heterotrophic organism, trout involved greater trophic conversions, thermal losses and energy incorporated into food, which explains its lower relative yield. However, it is important to note that, while the energy productivity of both organisms increased over time, SEC (MJ/kg) also showed an upward trend, particularly in trout (Fig. 61). This behavior contradicts efficiency expectations and creates uncertainties regarding the net energy balance in advanced stages of the production cycle.

In the case of trout, the sustained increase in SEC suggests that most of the energy was being consumed without being proportionally translated into biomass, which could be due to factors such as a reduction in the feed conversion rate, lower thermal

efficiency of the system, or accumulation of physiological stress in adult fish. In lettuce, although the SEC also increased, the increment was at a slower rate, allowing its energy productivity to remain favorable. This pattern may indicate a uniform use of energy by vegetable crops, probably associated with a higher consistency in nutrient assimilation and photoconversion rates, independent of the season. This energy resilience of lettuce, together with its low SEC variability, reaffirms its role as an energy-optimizing component within the integrated trout-lettuce system.

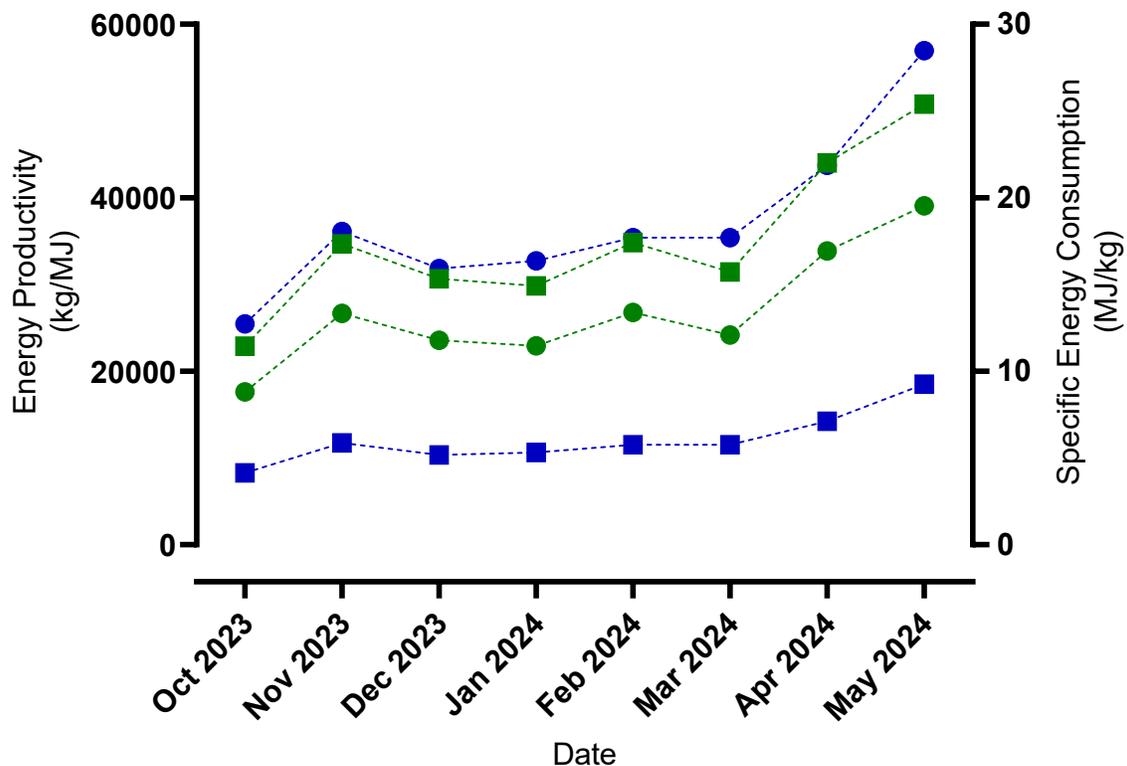


Figure 61. History of Energy Productivity (represented in square) and Specific Energy Consumption (represent in circle), for trout (blue line) and lettuce production (green line) in aquaponic system.

5.5.5 Nutrient Emissions: Acidification and Eutrophication Potentials

An Acidification Potential analysis (AP), measured in kilograms of SO₂-equivalent per kilogram of product, revealed an increasing trend in both components of the

system, although the magnitude of the increment differed. In the case of trout, AP values increased from 0.05 to 0.12 kg SO₂-eq/kg. This increment can be attributed to the cumulative rise in gaseous emissions resulting from nitrogen metabolism, particularly by the conversion of compounds such as ammonium (NH₄⁺) into Nitrates (NO₃⁻) via nitrification processes. Lettuce, on the other hand, exhibited higher AP values, ranging from 0.33 to 0.59 kg SO₂-eq/kg, exhibiting a lower temporal slope. This behaviour could be related to the initial phase of the hydroponic system when a supplementary nutrient foliar containing relatively high-impact inputs (e.g. phosphoric acid) was used. Regarding Eutrophication Potential (EP), which is measured in kg PO₄³⁻-eq/kg, the data reflected a higher environmental pressure in the trout component, with values ranging from 0.18 to 0.29 kg PO₄-eq/kg, that were increasing over time (Fig. 62). This indicates an accumulation of nutrients susceptible to leaching or discharge, particularly Phosphorus and Nitrogen, resulting from excreta and uneaten food. In the case of lettuce, the EP was considerably lower, with values ranging from 0.025 to 0.06 kg PO₄-eq/kg. This demonstrates its role in mitigating the eutrophic load of the system by assimilating the nutrients. These results support the hypothesis that including plant species such as lettuce in coupled aquaponic systems contributes to the efficient use of water and nutrients and acts as a biological filter, significantly reducing the systems environmental load in terms of eutrophication.

5.5.6 Water Use Indicators: Efficiency and Scarcity

The evolution of water use efficiency (WUE, kg/m³) and water intensity (WI, m³/kg) for lettuce and trout revealed divergent patterns that question the metrics traditionally used to assess the sustainability of integrated systems. While WUE increased steadily for both vegetables and fish, which in principle would indicate an improvement in yield per unit of water, this indicator alone was not able to capture the growing absolute water demands evidenced by the parallel increase in WI (Chairat et al., 2025).

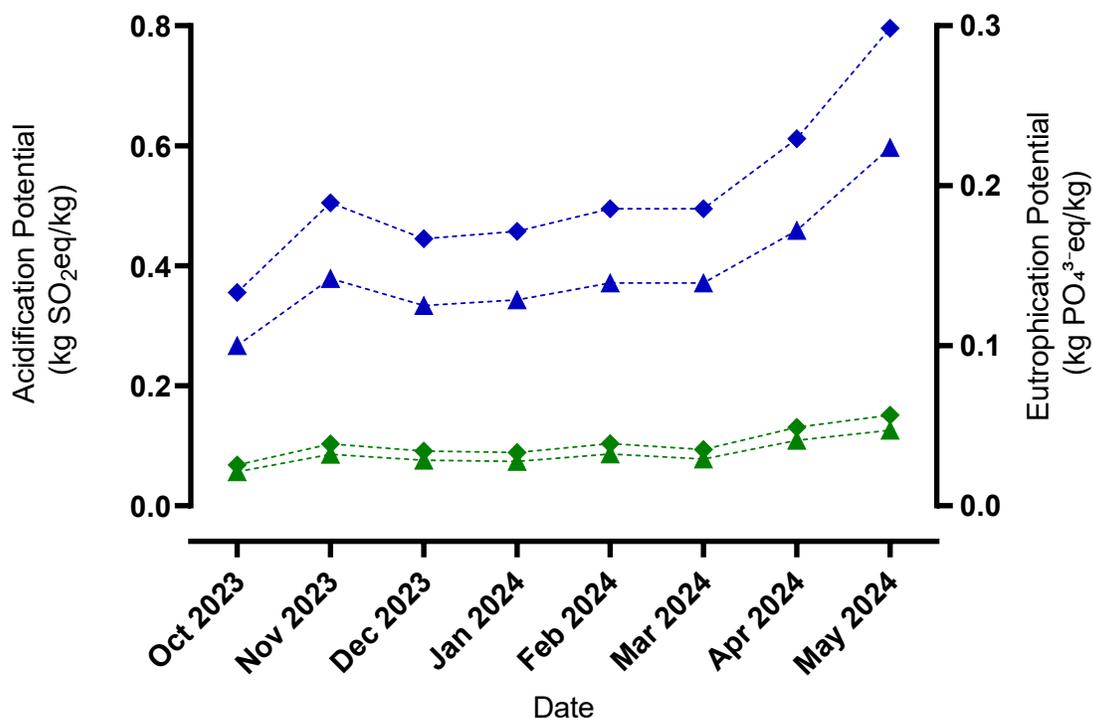


Figure 62. Temporal trends in Acidification Potential (represented in triangles) and Eutrophication Potential (represented in rhombus) for trout (blue line) and lettuce production (green line) in aquaponic system.

In the case of lettuce, the maximum efficiency, of close to 99 kg/m³, was achieved in May 2024, which seems a highly positive figure from a technical point of view. However, the highest water intensity value (10.08 m³/kg) was also reported for that same month, implying that the water required per unit of production had increased. This paradox between high efficiency and high intensity suggests that although the system was more productive per unit of volume, it was also more demanding in absolute terms per kilogram produced, possibly due to the lengthening of the production cycle, higher evapotranspiration rates, or overfeeding of the system during the advanced stages of cultivation.

In trout, this contradiction was also evident: although their WUE value increased two times during the period (from ~18 to ~36 kg/m³), the increment of WI from 1.85 to 4.13 m³/kg, originated an increment of 123% in water pressure per unit of product.

This discrepancy may be due to an imbalance between water supply and specific growth, probably linked to the accumulation of solids or fluctuations in water quality, indirectly affecting feed conversion (Fig. 63). From a critical perspective, these results call for a simultaneous assessment of relative (efficiency) and absolute (intensity) indicators. Measuring WUE alone could create a false perception of sustainability if it is not considered that, at the same time, the system is consuming more water per kilogram of trout and lettuce produced. This is particularly relevant in arid and semi arid regions such as in the northern of Chile, where efficient water management cannot be separated from the control of absolute water flows or the design of efficient reuse strategies.

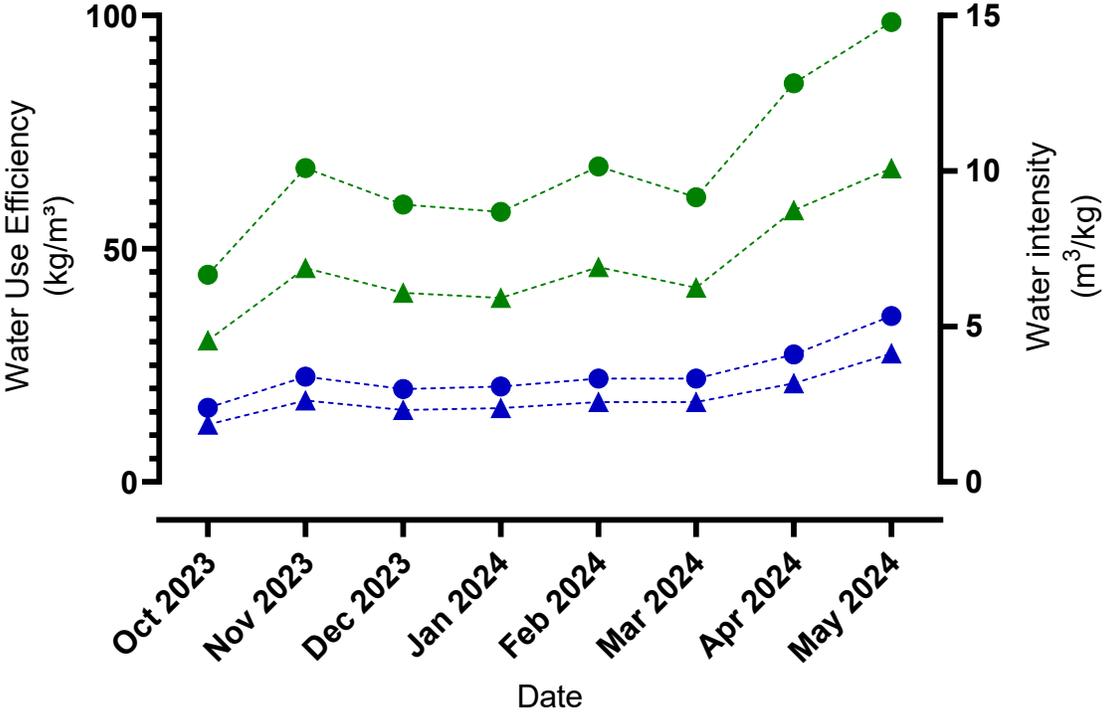


Figure 63. Evolution of Water Use Efficiency (represented in circle) and Water Intensity (represented in triangle) for trout (blue line) and lettuce production (green line) in aquaponic system.

5.5.7 Energy Productivity Analysis

The values obtained for specific energy consumption (MJ/kg) revealed systematic differences between the two species. In the case of trout, SEC values ranged from 490 to 890 MJ/kg over nine months, increasing progressively towards the end of the period. This upward trend is associated with the increased biomass and higher system maintenance requirements, including oxygenation and water recirculation. In contrast, lettuce SEC values remained more stable, fluctuating between 145 and 255 MJ/kg, suggesting a higher energy efficiency in plant production under controlled hydroponic conditions.

In terms of cumulative energy demand (CED), which incorporated both direct and indirect energy flows (e.g. material inputs, transport or infrastructure-related energy), patterns consistent with SEC were observed. CED for trout exceeded 500 MJ/kg from the third month onwards, whereas for lettuce it remained between 110 and 245 MJ/kg, reflecting a lower energy cost throughout its production life cycle (Fig. 64). These results suggest that, from an energy perspective, lettuce was a more efficient and less resource-intensive crop. However, it is important to note that integrating both crops allowed for the cross-utilisation of energy and nutrients, which partially mitigated the total energy impact of the coupled system.

5.5.8 Environmental assessment: Energy and Carbon Footprint

Figure 65 illustrates the monthly evolution of Specific Energy Consumption (SEC) and Global Warming Potential (GWP) for trout and lettuce. The results show significant differences in the environmental intensity of both components of the system.

The environmental intensity of trout production was consistently higher, with GWP values ranging from 29.5 to 58.7 kg CO₂eq/kg and SEC values ranging from 17.5 to 28.3 MJ/kg. By contrast, lettuce exhibited considerably lower values, with a GWP ranging from 6.2 to 12.4 kg CO₂eq/kg and an SEC varying from 6.4 to 19.1 MJ/kg. This suggests that the carbon footprint of trout was between three and five times

higher than that of lettuce, while its energy intensity was between 1.3 and 2.8 times higher depending on the analyzed month.

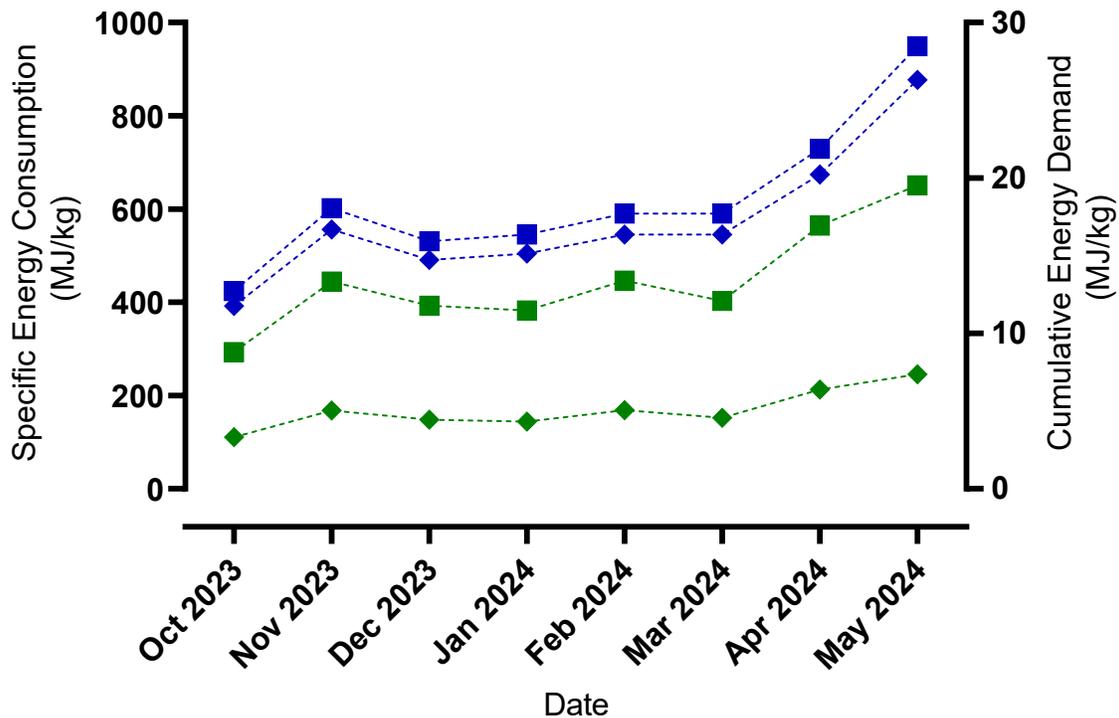


Figure 64. Evolution of the Specific Energy Consumption (represented in square) and Cumulative Energy Demand (represented in rhombus) for trout (blue line) and lettuce production (green line) in aquaponic system.

Regarding the variation in time, a significant-increment of the environmental impact of trout production was observed in April and May 2024, coinciding with the seasonal temperature changes. This sudden rise in GWP and SEC suggests a direct correlation between electricity usage and carbon emissions, particularly towards the end of the production cycle when biomass and feed consumption were at their peak.

In comparison, the environmental performance of lettuce remained more stable throughout the period. Although the SEC increased slightly in the early months, the GWP remained within a narrower range. This stability reinforces the idea that

hydroponic vegetable production requires less energy and generates fewer emissions than aquaculture. These results support the hypothesis that energy consumption (SEC) is the main determinant of climate impact (GWP) in aquaponic systems. The strong correlation between the two indicators, particularly regarding to trout, corroborates prior observations in the literature by Greenfeld et al. (2022) and by Goddek et al. 2015), which suggested that electricity consumption associated with life support systems for aeration, heating and pumping, dominates the environmental profile (Fig. 65).

From a sustainability perspective, lettuce acts as an environmental buffer within the coupled system, offering a much more energy- and carbon-efficient route to biomass production. However, to reduce the overall impact of the system, it is key to advance in developing new energy efficiency strategies, such as on-demand aeration, pulsed hydraulic flow, or even the integration of renewable energies to supply aquaculture units.

5.6 Discussion

A summary of the main experimental results obtained for the environmental performance of a trout – lettuce aquaponic integrated system is depicted in Table 4. The data were obtained from a gate-to-gate Life Cycle Assessment (LCA) applied to evaluate the evolution of the five environmental indicators: Water Use Efficiency (WUE), Specific Energy Consumption (SEC), Cumulative Energy Demand (CED), and Global Warming Potential (GWP), over a nine-month production cycle.

Lettuce Water Use Efficiency (WUE) values observed in the analyzed aquaponic system ranged from 61 to 101 kg/m³, which falls within the expected range of 50 to 120 kg/m³ reported by Barbosa et al. (2015).

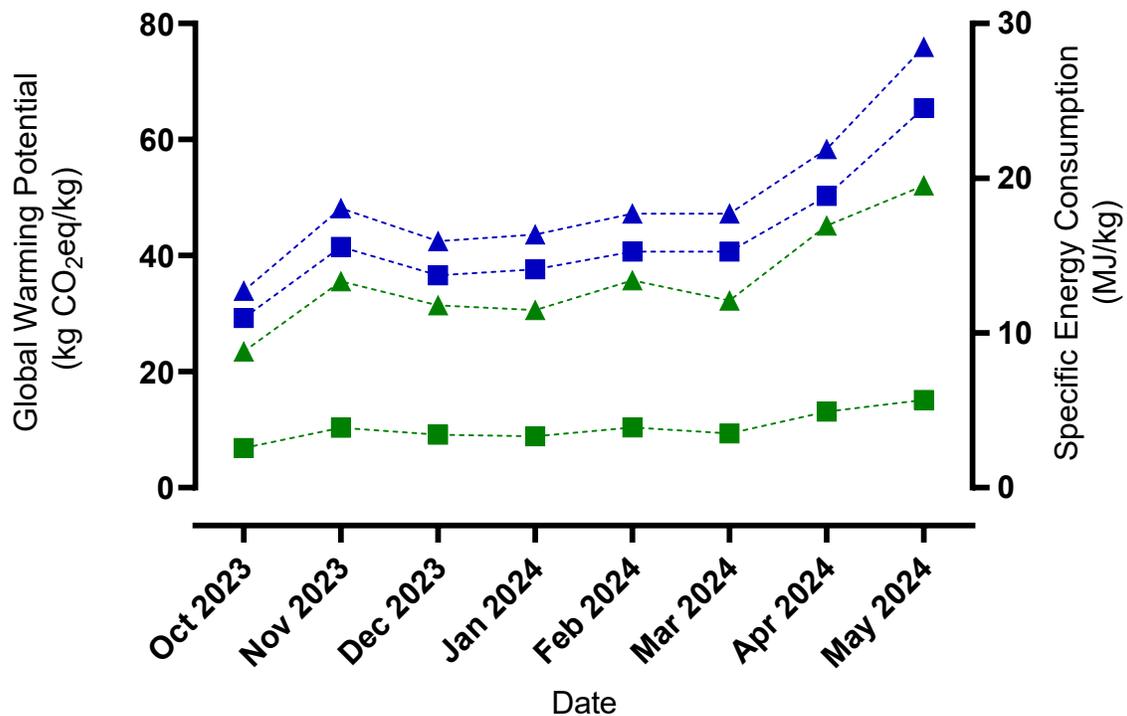


Figure 65. Evolution of Specific Energy Consumption (represented in triangle) and Global Warming Potential (square) for trout (blue line) and lettuce production (green line) in aquaponic system.

Goddek et al. (2015) and by Somerville et al. (2014). These values suggest that the plant system is highly efficient in terms of converting water into biomass, which is favored by the controlled conditions of the greenhouse and the adequate management of the recirculating aquaculture system (RAS) (Table 14).

For trout, the values of the WUE were between 18 and 36 kg/m³, which are also within the reported range in the scientific literature (10 to 35 kg/m³) (Bordignon et al., 2022a). This result indicates that the water yield of the animal component is sufficient, although occasional increases could be associated with variations in harvested biomass or differences in the stocking density. These aspects have been identified as determinants in other aquaponic studies (Rouphael and Colla, 2005).

SEC values for lettuce (145–255 MJ/kg) are within the expected range (150–300 MJ/kg), indicating an energy consumption that was consistent with technician

hydroponic systems. Similarly, the SEC values for trout (490 to 890 MJ/kg) were within the reported range in the literature (450 to 1000 MJ/kg). However, the upward trend in the final months suggests an increment in the energy requirements, possibly due to the intensive use of aerators or higher thermal requirements during the final stage of fattening. This is consistent with previous reported values (Forchino et al., 2018; Maucieri et al., 2018a).

Regarding CED, the lettuce results (110 to 245 MJ/kg) and the trout data obtained (500 to 800 MJ/kg) were in agreement with the previous published values (100 to 300 MJ/kg for vegetables and 500 to 900 MJ/kg for fish), thus validating the overall system energy performance. However, the comparative efficiency of the plant system versus the animal system remained unchanged, reaffirming the lower energy intensity of lettuce cultivation under this mixed production scheme.

The values of global warming potential (GWP) associated with lettuce production in this study ranged from 6.2 to 12.4 kg CO₂-eq/kg, which was within the expected range of 5 to 15 kg CO₂-eq/kg reported by (Bordignon et al., 2022b). This indicates that the vegetable has a moderate climate impact. This is a particularly relevant result considering that the production of a healthy crop takes place in closed, controlled systems where losses can be minimized and inputs optimized.

For trout, the GWP was found to range from 29.5 to 58.7 kg CO₂-eq/kg, which is also within the expected range (25 to 70 kg CO₂-eq/kg) and consistent with the findings of studies such as those by Roque d'Orbcastel et al. (2009). While these values are significantly higher than those for the plant component, they reflect the intensive nature of fish farming and the associated energy use for recirculation, feeding, and controlling physicochemical parameters. present a summary of the main environmental indicators obtained from the present integrated trout-lettuce aquaponic system and those values reported in the scientific literature that are relevant from a sustainable perspective.

Table 14. Summary of the environmental indicators assessed in this experience of lettuce-trout growth in an integrated aquaponic system versus those reported in literature.

Indicator	Lettuce	Trout	Expected range Lettuce (literature)	Expected range Trout (literature)	Literature
WUE (kg/m ³)	61 to 101	18 to 36	50 to 120	10 to 35	(Barbosa et al., 2015; Goddek et al., 2015; Somerville et al., 2014)
WI (m ³ /kg)	1.0 to 1.3	1.85 to 4.1	0.8 to 2.0	2.0 to 6.0	(Forchino et al., 2018; Maucieri et al., 2018b)
SEC (MJ/kg)	145 to 255	490 to 890	150 to 300	450 to 1000	(Bordignon et al., 2022c; Roque d'Orbcastel et al., 2009)
CED (MJ/kg)	110 to 245	500 to 800	100 to 300	500 to 900	
GWP (kg CO ₂ -eq/kg)	6.2 to 12.4	29.5 to 58.7	5 to 15	25 to 70	

5.7 Preliminary conclusions

The present study assessed the environmental sustainability of a coupled trout–lettuce aquaponic system by estimating energy and water consumption through a Life Cycle Assessment (LCA). Empirical data were collected during a nine-month period, encompassing one complete trout production cycle and eight lettuce crop cycles. Inputs such as smolt transportation, fish feed, electricity, water, seedlings, substrates, and greenhouse materials were accounted for, as well as outputs including trout biomass, lettuce yield, residual water, and carbon emissions.

The multifunctionality of the system was addressed using mass-based, economic, and system expansion allocation approaches to compare environmental burdens between components. Life Cycle Assessment indicators in the lettuce subsystem had significantly lower environmental impacts (WUE: 61–101 kg/m³; SEC: 145–255 MJ/kg; GWP: 6.2–12.4 kg CO₂-eq/kg) than those in the trout subsystem (WUE: 18–36 kg/m³; SEC: 490–890 MJ/kg; GWP: 29.5–58.7 kg CO₂-eq/kg). Therefore, the

coupled lettuce-trout system contributed to reduce energy, water and greenhouse gas emissions (GHG) by the trout nutrients recycling. Overall, the findings validate the environmental feasibility of integrated aquaponics and provide essential insight for future design improvements aimed at increasing system efficiency and sustainability. Healthy human food production, such as lettuce and trout, in an integrated coupled aquaponics system, can be a relevant-sustainable alternative to reduce the global change, especially suitable for arid and semi-arid zones.

Allocation scenarios, based on mass, economic value, and system expansion, consistently highlighted the stark environmental contrast between components, with lettuce production displaying lower Global Warming Potential (GWP), Specific Energy Consumption (SEC), and Water Use Efficiency (WUE) per kilogram. These findings reaffirm the viability of aquaponics as a sustainable alternative for food production in resource-limited regions, while also pointing toward the need for targeted innovations in energy optimization, waste valorization, and nutrient management. These insights can inform both technological improvements and policy directions for scaling aquaponics in a sustainable manner.

CHAPTER 6

6 Conclusions

The numerical-experimental study of energy and water consumptions for a sustainable aquaponic growth of trout – lettuce allows to formulate the conclusions drawn for each hypothesis and goal.

Energy-environmental sustainability in the growth of trout and lettuce, in a recirculating aquaponic system, improved when the transport phenomena of fluids, heat and matter in the productive cycle were known and characterized.

- Mass balances demonstrated that understanding the generation, transformation, and removal of nitrogen compounds was essential for maintaining water quality and supporting stable plant–fish integration. The explicit coupling of nitrogen production from feeding, biofilter nitrification capacity, and nutrient uptake by lettuce enabled the system to recycle internal resources more efficiently, reducing the need for external inputs and minimizing pollutant accumulation. Quantifying the dynamics of TAN, nitrite, and nitrate revealed the key regulatory role of the biofilter and validated the importance of controlling operational variables such as pH, temperature, and recirculation rate.
- Energy balances revealed that the distribution and transport of thermal energy across the hydraulic circuit significantly influenced metabolic rates, oxygen dynamics, and system performance. Identifying the thermal gradients and hydraulic inefficiencies allowed to optimize aeration, pumping, and water distribution, with a low Specific Energy Consumption (SEC). The numerical simulations of the fluid mechanical and heat transfer evolution in the hydroponic bed, and the results of the evolution of velocity and temperature distribution revalidated against experimental data, confirmed that energy use efficiency improved when flow regimes were adjusted to reduce dead zones, enhance oxygen transfer, and stabilize temperature within optimal biological ranges.

- Computational fluid dynamics (CFD) successfully described internal flow patterns and helped explain nutrient distribution toward the roots, demonstrating the potential of integrating sensor-based measurements with predictive models. Limitations included the absence of biological and chemical processes in the mathematical formulation and the lack of quality assessments of the lettuces and water. Future work should incorporate organoleptic and nutritional analyses, pollutant monitoring, and year-round experiments, while refining computational models to include dynamic biological factors such as nutrient uptake, respiration, and oxygen consumption.

The integrated knowledge of engineering, biology and aquaculture in water, energy and nitrogen compounds flow prediction allowed to obtain the following conclusions:

- Improved productivity was achieved by linking hydrodynamic behavior, nutrient dynamics, and biological performance. The predictive models identified the flow regimes, oxygen distribution patterns, and nutrient availability conditions that maximized growth rates in both trout and lettuce. This integration enabled the adjustment of water recirculation rates, feeding strategies, and hydraulic configurations, resulting in higher biomass yields and more stable growth throughout the production cycle.
- The enhanced quality of the recirculating trout-lettuce aquaponic system was one of the relevant findings to generate original results for the efficient trout and lettuce growth.
- The integrated information of nitrogen transformation pathways, thermal behavior, and biological demands, contributed to generating original insights for the coupled aquaponic system.
- The inclusion of nitrification kinetics, oxygen consumption patterns, and thermal gradients into the predictive framework allowed the system to maintain optimal water quality, controlling TAN, nitrate, and dissolved oxygen within safe thresholds, while improving lettuce nutrient uptake and fish welfare.
- Generation of original results for productive scaling up emerged from the integration of numerical simulations and experimental data. The validated models

demonstrated how changes in biomass density, hydraulic loading, and energy distribution affect the stability of the system at larger scales. This permitted the proposal of evidence-based design criteria for future aquaponic units, including biofilter sizing, flow distribution strategies, residence times, and water-energy optimization approaches.

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APPENDIX A: Paper published

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Effect of hydraulic configuration on lettuce growth in hydroponic bed using Deep water culture technique (DWC)

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Influence of lettuce array

ABSTRACT

Experiments and computational modeling were developed to determine the effect of different types of hydraulic configurations on water quality variables to improve growth of lettuce in hydroponic beds. The variants in the hydraulic configurations consider water recirculation in hydroponic modules using Deep Water Culture technique (DWC), for continuous (CWF) and pulsatile water flow (PWF) using either one or three water flow inlets (TWF). These data were used to generate fluid mechanics and heat transfer models for the described hydraulic configurations to assess the effect of the hydraulic configuration on lettuce growth. The results obtained from the mathematical model by the finite volume method allowed to explain the influence of water flow and temperature on the rate of growing for lettuce during summer and autumn in the southern hemisphere. The main findings obtained from the hybrid numerical – experimental model to achieve high lettuce yield were that the number of water inlets has an effect on influenced nutrient transport and water quality variation, where the variant with three water inlets (TWF), and the climatic condition for autumn achieve better plant growth performance than summer. Computational modelling of fluid mechanics and heat transfer allowed to predict the variation of water quality variables in DWC bed, being a suitable technique with a high potential for achieving new accurate agriculture standards.

1. Introduction

Globally, in the last five the harvested area of lettuce and chicory has remained around an average value of 1,213,000 ha. During the last decade the production of lettuce and chicory has been growing at an average annual rate of 2.87 %, reaching 27,011,747 tons in 2021 (FAOSTAT, 2022).

The hydroponic farming approach is considered as a sustainable and viable option for minimizing water usage compared to traditional techniques. Particularly, due to the climate change current scenery, many regions are suffering from soil degradation and scarce water resources (Velazquez-Gonzalez et al., 2022), and hence horticultural hydroponic farming offers significant advantages. Notably, this farming technique is environmentally friendly, as it does not rely on pesticides and utilizes a significantly reduced quantity of water (Casey et al., 2022). Conventional farming, on the other hand, faces several

challenges, mainly related to soil quality (Kannan et al., 2022) and climate (Holmes et al., 2019).

There are several alternative horticultural farming systems besides hydroponics, such as aquaponics (combining aquaculture and agriculture) and aeroponics (utilizing a mist environment for plant growth), along to the substrate culture like cultivating crops with fungi (Frasetya et al., 2021). Nonetheless, hydroponic farming has gained a significant attention due to its exceptional nutrient management and high yields (Pineda-Pineda et al., 2017).

Hydroponics is generally developed in rectangular beds, called raceways in aquaculture, using the DWC (Deep Water Culture) technique, or NFT (Nutrient Film Technique). DWC hydroponic systems are a type of stationary system that utilize floating rafts to support plants (Kozai et al., 2022). These rafts floats on the hydroponic beds which are filled with nutrients-rich water. On the other hand, NFT hydroponic systems consist of grow channels that circulate a layer of nutrient

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APPENDIX B: Submitted manuscripts

1.-

Action 	 Manuscript Number 	Title 	Date Submission Began 	Status Date 	Current Status 
Action Links	NEXRES-D-25-00290R1	Model-Based estimation of Water Use and Irrigation Water Requirements in Lettuce production in Semi-Arid Regions	Jul 16, 2025	Aug 08, 2025	Under Review

2.-

Action 	 Manuscript Number 	Title 	Initial Date Submitted 	Status Date 	Current Status 
Action Links	AQUACULTURE-D-25-05019	Environmental performance of Trout – Lettuce Aquaponic integrated System measured: A Life Cycle Assessment.	10/22/2025	10/30/2025	With Editor

APPENDIX C: Diffusion at National and International Congress

- C. Jana, E. von Brand, V. Arancibia, N. Pérez, C. Concha, C. Cortés, C. Merino, A. Durán, M. Pino, Cultivos Integrales AgroAcuícola, 1er Congreso Internacional Agroacuícola Producción y Nutrigenómica en Plantas y Animales, como Alternativas para la Sustentabilidad Alimenticia y Salud, 7 al 16 mayo 2025 – Universidad Popular de La Chontalpa – Cárdenas, Tabasco – México.
- C. Jana, E. von Brand, V. Arancibia, N. Pérez, C. Concha, C. Cortés, C. Merino, A. Durán, M. Pino, Biointegrated Production System Technology and a International Network, 14th Annual Aquaponic Conference. September 15 to 17, 2025 – College Park, Maryland – USA.
- C. Jana, E. von Brand, V. Arancibia, N. Pérez, C. Concha, C. Cortés, C. Merino, A. Durán, M. Pino, Biosolids mineralization and biofilter microbiota associated to pilot scale aquaponic. Systems rearing rainbow trout, river prawn and lettuces, 13th Annual Aquaponics Conference, Tulsa, USA, September 12th-15th, 2024.
- C. Cortes, G. Merino, Lettuce crop performance (l. Sativa), using different water aeration at DWC. Technique, Latin American and Caribbean Aquaculture 2024, Medellin, Colombia. September 24th-27th, 2024.
- C. Cortes, G. Merino, Yield and growth of lettuce (l. Sativa), in DWC hydroponics vs. Aquaponics, in the southern hemisphere during the summer season, Latin American Caribbean Aquaculture 2024, Medellin, Colombia. September 24th-27th, 2024.
- C. Cortes, C. Jana, V. Arancibia, V. Pizarro, Content of Polyphenols and antioxidant capacity of lettuces produced under two production systems, 22nd World Congress of Food Science and Technology 2024. September 8-12th, 2024.