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Innovative and Sustainable Cultivation of Brassica *Eruca vesicaria* with Reuse of Wastewater from the Production of *Squalius alburnoides* by Decoupled Aquaponics

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ABSTRACT

The growth of the world population represents a great challenge for an adequate response to sustainable food production. Unfortunately, the conventional approach to agriculture, adopted in response to this challenge, entails adverse consequences for both the environment and consumer health. To control and/or reduce these consequences, various sustainable plant production techniques have been employed with great success.

Aquaponics is a practice that combines hydroponics with aquaculture, distinguishing itself from conventional practices through the more efficient use of water, as the essential nutrients for plant matrix growth come from fish production wastewater. This practice can be established in a coupled or decoupled manner (without a water recirculation system between the hydroponic and aquaculture systems).

To meet the specific nutritional needs for sustainable plant production, this project proposes the implementation of a decoupled aquaponics system for the hydroponic production of rocket (*Eruca vesicaria* L.) using wastewater from the production of a native freshwater fish, Calandino (*Squalius alburnoides*), in an aquaculture system. The wastewater will be monitored in terms of physicochemical and microbiological parameters, namely, pH, conductivity, temperature, dissolved oxygen concentration and aeration, ammonia and nitrate concentrations, total nitrogen and phosphorus, total coliforms, thermotolerant coliforms, *Escherichia coli*, and sulfite-reducing *Clostridium* spores. Additionally, the quality of aquaponics produced rocket will be evaluated through physiological growth characteristics and through its nutritional, chemical, and bioactive profile compared to commercial arugula.

The results revealed critical challenges that affected the performance of the aquaponic system, including suboptimal environmental and water quality conditions for both fish and plants. For the aquaculture component, high nitrite, phosphorus, and dissolved oxygen levels, along with high microbial contamination, compromised fish health and nutrient availability. Similarly, the hydroponic component experienced growth challenges due to fluctuating environmental factors such as temperature, humidity, pH, and electrical conductivity, which negatively impacted rocket plant development. Despite these challenges, the aquaponic system showed potential for producing nutrient-dense crops, with the microbial load in fish wastewater underscoring the need for effective water treatment and microbial management.

This study emphasizes the importance of rigorous control over key environmental parameters in aquaponics, highlighting the need for optimized systems that balance the specific requirements of both the aquaculture and hydroponic components. By fine-tuning

nutrient concentrations, improving water quality management, and implementing better climate control, aquaponics systems can enhance both fish health and plant productivity, offering a sustainable alternative to conventional agriculture. The findings contribute valuable insights into refining aquaponic practices for improved sustainability and productivity in food production systems.

Keywords: decoupled aquaponics, rocket, calandino, wastewater, nutritional profile.

RESUMO

O crescimento da população mundial representa um grande desafio para uma resposta adequada para a produção sustentável de alimentos. Infelizmente, a abordagem convencional da agricultura, adotada em resposta a este desafio, acarreta consequências adversas tanto para o meio ambiente como para a saúde do consumidor. De forma a controlar e/ou diminuir estas consequências, várias técnicas de produção sustentável de plantas têm vindo a ser empregues com grande sucesso.

A aquaponia é uma prática que conjuga a hidroponia com a aquacultura, distinguindo-se das práticas convencionais pelo uso mais eficaz da água, uma vez que os nutrientes essenciais para o crescimento da matriz vegetal são oriundos das águas residuais de produção de peixe. Esta prática pode ser estabelecida de forma acoplada ou desacoplada (sem sistema de recirculação de água entre o sistema hidropónico e de aquicultura).

Com o objetivo de satisfazer as necessidades nutricionais específicas para a produção vegetal de forma sustentável, este projeto propõe a implementação de um sistema de aquaponia desacoplado para a produção em sistema hidropónico de rúcula (*Eruca vesicaria L.*) através de águas residuais de produção de um peixe nativo, o bordalo (*Squalius alburnoides*) em sistema de aquacultura. As águas residuais serão monitorizadas em termos de parâmetros físico-químicos e microbiológicos, nomeadamente, pH, condutividade, temperatura, concentração de oxigénio e oxigenação, concentração de nitrato de amónia, nitrogénio total e fósforo, coliformes totais, coliformes termotolerantes, *Escherichia coli* e esporos de *Colstridium* sulfito-redutores. Ainda, a qualidade da rúcula produzida em aquaponia será avaliada através de características fisiológicas de crescimento, mas também através do seu perfil nutricional, químico e bioativos comparada com a rúcula comercial.

Os resultados mostraram desafios críticos que afetaram o desempenho do sistema aquapónico, incluindo condições subótimas de ambiente e qualidade da água tanto para os peixes como para as plantas. Para o componente da aquacultura, os baixos níveis de nitritos, fósforo e oxigénio dissolvido, juntamente com a alta carga microbiana, comprometeram a saúde dos peixes e a disponibilidade de nutrientes. Da mesma forma, a componente hidropónica enfrentou dificuldades de crescimento devido a fatores ambientais flutuantes, como temperatura, humidade, pH e condutividade elétrica, que afetaram negativamente o desenvolvimento da rúcula. Apesar destes desafios, o sistema aquapónico revelou potencial para produzir culturas de plantas ricas em nutrientes, com a carga microbiana nas águas

residuais dos peixes a sublinhar a necessidade de um tratamento eficaz da água e gestão microbiana.

Este estudo enfatiza a importância de um controlo rigoroso sobre os parâmetros ambientais chave na aquaponia, destacando a necessidade de sistemas otimizados que equilibrem os requisitos específicos dos componentes da aquacultura e da hidroponia. Ao ajustar as concentrações de nutrientes, melhorar a gestão da qualidade da água e implementar um melhor controlo das condições climátiacs, os sistemas aquapónicos podem melhorar tanto a saúde dos peixes como a produtividade das plantas, oferecendo uma alternativa sustentável à agricultura convencional. Os resultados fornecem *insights* valiosos para refinar as práticas em aquaponia, melhorando a sustentabilidade e produtividade nos sistemas de produção de alimentos.

Palavras-chave: aquaponia desacoplada, rúcula, bordalo, águas residuais, perfil nutricional.

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LIST OF ABBREVIATIONS

BGLB - Brilliant Green Lactose Bile Broth

BHT - Butylhydroxytoluene (solution)

CFU - Colony-Forming Units

cm - Centimeter

dw - Dry Weight

EC - Electrical Conductivity

EU - European Union

FAMEs - Fatty Acid Methyl Esters

FID - Flame Ionization Detector

g - Gram

GC - Gas Chromatography

H2SO4 - Sulfuric Acid

HPLC - High-Performance Liquid Chromatography

ISO - International Organization for Standardization

Kg - Kilogram

KI - Potassium Iodide

KI - Potassium Iodide

LST - Lauryl Sulfate Tryptone

m - Molar

mL - Milliliter

mm - Millimeter

MnSO4 - Manganese (II) Sulfate

MPN - Most Probable Number

mS - Millisiemens

Na2S2OQ - Sodium Thiosulfate

NaOH - Sodium Hydroxide

N – Nitrogen

NOQ^{-} - Nitrate Ion

ºC - Degrees Celsius

TSC - Tryptone Soy Casein Agar Base

µL - Microliter

µm - Micrometer

mg - Milligram

1. Introduction

The exponential growth of the global population, now surpassing 7.2 billion and projected to reach 9.6 billion by 2050, presents a challenge, especially as more than three-quarters of this population will dwell in urban areas (Malik, 2013). This migration to cities strains the conventional use of land, traditionally allocated for soil-based agriculture, as escalating urban needs for housing and infrastructure infringe upon these fertile grounds (Alexandratos & Bruinsma, 2012).

However, the traditional practices of farming, particularly intensive animal protein production, fluctuating energy costs, the ominous shadow of climate change, and the big problem of pollution pose serious challenges to meet this escalating demand. Moreover, compounding issues like the diminish of arable land, dwindling freshwater reservoirs, the degradation of soil quality, and the depletion of essential nutrients heighten the urgency to rectify the sustainability deficits in our global food systems (Goddek et al., 2015).

In response to these complex challenges, the integration of aquaculture and hydroponics emerges as a beacon of hope. Initially prevalent in arid regions, technological advancements have propelled aquaponics beyond these confines, heralding it as an efficient, sustainable method. This innovative approach unites aquaculture and hydroponics, employing mineral transfers to facilitate effective nutrient recycling while curbing water usage through smart recirculation techniques (Ragnarsdóttir, 2008). The reuse of wastewater plays a pivotal role in ensuring the sustainability of these integrated aquaponic systems (Goddek et al., 2019).

Thus, this project aims to implement a decoupled aquaponics system for cultivating rocket (*Eruca vesicaria*) in a hydroponic system, using wastewater from a native fish, Calandino (*Squalius alburnoides*), in an aquaculture system. The wastewater will be monitored for physical-chemical and microbiological parameters, including pH, conductivity, temperature, oxygenation, saturation, ammonia nitrate, total nitrogen, and phosphorus concentrations, total coliforms, thermotolerant coliforms, *Escherichia coli*, and sulphite- reducing clostridia spores. Furthermore, the quality of rocket produced through aquaponics will be assessed based on physiological, nutritional, chemical, and bioactive profiles, and compared with commercial rocket.

2. State of the art

2.1. Conventional and non-conventional agricultural systems

Conventional agricultural systems rely heavily on chemical inputs, promote monocultures, and aim to maximize short-term yields. Conversely, non-conventional systems like hydroponics, vertical farming, urban farming, agroecology, permaculture, and organic production employ sustainable approaches, emphasizing crop rotation, natural fertilizers, and biological pest control methods. While conventional systems might sometimes yield higher immediate outputs, non-conventional methods tend to offer greater long-term resilience, preserving soil fertility, reducing reliance on costly external inputs, and minimizing detrimental environmental impacts, these innovative approaches aim to address these challenges while promoting environmental protection and long-term food security (Sumberg & Giller, 2022).

An emerging unconventional agricultural system is the practice called aquaponics, the integration of aquaculture and hydroponics appears as a beacon of hope. Initially predominant in arid regions, technological advances have pushed aquaponics beyond these limits, announcing it as an efficient and sustainable method.

This innovative approach unites aquaculture and hydroponics, employing mineral transfers to facilitate effective nutrient recycling while reducing water use through smart recirculation techniques (Ragnarsdóttir, 2008). However, there are limitations to consider, especially in high-yield hydroponic systems that rely heavily on industrial and mining-derived nutrients. These systems consume significant energy and finite resources, highlighting the need for more sustainable alternatives. However, rapid advances in design and practices have propelled aquaponics into large-scale industrial production, significantly enhancing production capabilities and operational efficiency (Turcios & Papenbrock, 2014).

2.1.1. Non-conventional agricultural systems

2.1.1.1. Aquaponics

While traditional hydroponics requires mineral fertilizers to provide plants with the nutrients they need, aquaponics systems use available fish water, rich in fish waste, for plant growth. Another advantage of this combination is that there is no need to regularly replace concentrated fish water with fresh water to remove excess nutrients, which is common in aquaculture systems. This system provides a symbiosis of fish, microorganisms, and plants

and promotes sustainable use, including recycling of water and nutrients, as illustrated in **Figure 1**. In this synergistic interaction, the respective ecological weaknesses of aquaculture and hydroponic farming are transformed into strengths. This combination significantly minimizes the need for nutrient inputs and waste outputs, unlike individual systems (Goddek et al., 2015)

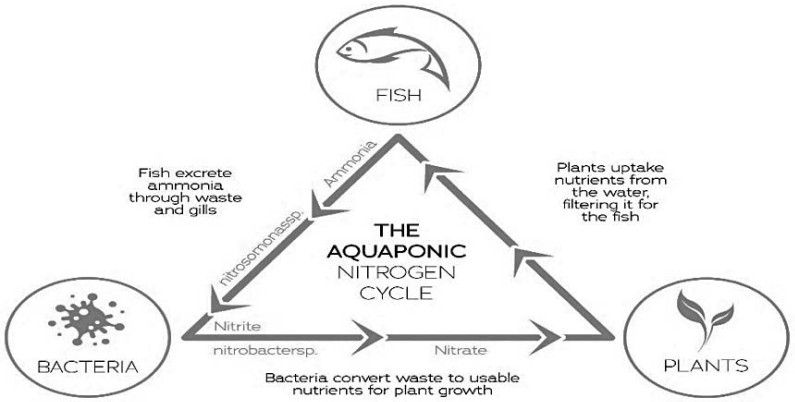


Figure 1. Symbiotic aquaponic (Goddek et al., 2015).

Plants require macronutrients such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulphur (S), magnesium (Mg) and micronutrients such as iron (Fe), chlorine (Cl), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), essential for their growth. Hydroponic fluids contain these elements in well-defined proportions, except for C, H, and O, which are obtained from air and water. In an aquaponics system, the phytonutrient input from the aquarium includes dissolved nutritious fish waste. It consists of both soluble and solid organic compounds that dissolve in water in ionic form and are assimilated by the plant. Micronutrient and macronutrient concentrations must be monitored to maintain proper plant growth. Periodically, it may also be needed to add some of the nutrients or even adjust their concentration (Goddek et al., 2015).

2.1.1.2. Aquaculture

Aquaculture is the controlled cultivation and propagation of aquatic organisms, such as fish, shellfish, and plants, in controlled environments such as ponds, tanks, and marine enclosures for commercial, recreational, or conservation purposes. Continues to dominate aquatic food production in Asia and around the world (Tacon 2020). Currently, more than 91% of the world's aquaculture production (102.9 million tons in 2017) is produced in Asia, and total global aquaculture production exceeds global capture fishery production by more than 18.32 million tons. **Figure 2** describes the total global aquaculture and capture fisheries

production between 1950–2017. Furthermore, unlike most land-based agricultural food production systems, more than 95% of the world's aquaculture production is currently realized in developing countries, and production in these countries is growing at an average annual rate of 6.13%. In 2017, the total production value of aquaculture exceeds $250 billion, and the aquaculture sector supports a variety of aquatic plant and animal species that benefit from the production of unicellular Chlorella algae in indoor bioreactors (Tacon, 2020).

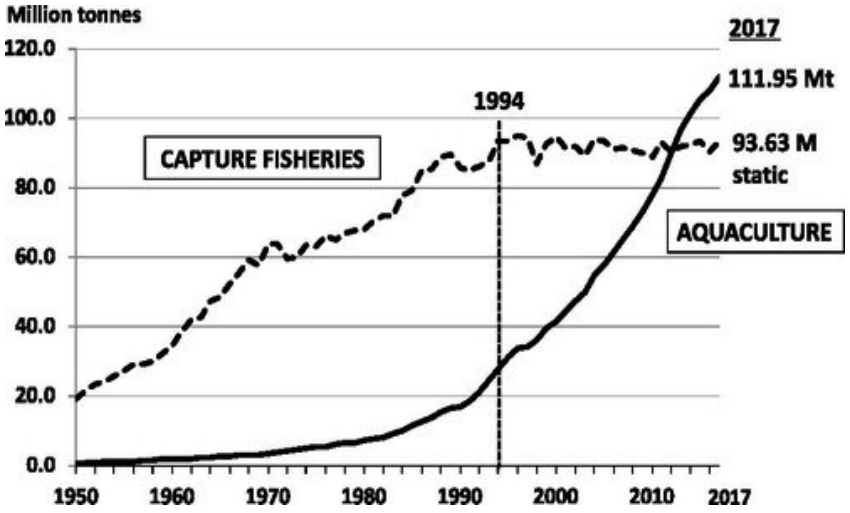


Figure 2. Total global aquaculture and capture fisheries production 1950–2017 (Tacon 2020).

2.1.1.3. Hydroponic

Today, the challenge of food security is a global concern, with global food production needing to increase by 70% over the next 40 years to meet the needs of more than 9.1 billion people by 2050. In parallel with urbanization and the development of new towns, urban farming systems, also known as hydroponic systems, are expanding the use of limited land areas suitable for farming and more rational use of water resources to create better farming opportunities. Hydroponic systems are a method of growing plants without the use of traditional soil and these systems are considered a viable solution to sustainable food supply in both developed and developing countries. Plants receive the nutrients they need directly from carefully prepared aqueous solutions, this solution circulates around the plant's roots, providing an optimal growing environment. The hydroponic approach allows for precise control over factors such as nutrient concentration, pH, and water distribution, allowing for efficient and controlled plant growth. This method is often used in agriculture and horticulture to increase crop yields while saving resources. Hydroponic systems are new cultivation techniques that do not use soil substrates and apply nutrient solutions in the presence of artificial supporting media. This provides an opportunity to reuse water and nutrients,

simplify the control of environmental fluctuations, increase production yields, and progressively prevent soil-borne diseases and pests (Chow et al., 2017). The advantages and limitations of hydroponic systems in comparison to soil-based culture are summarized in **Table 1.**

Table 1. Advantages and limitations of hydroponic systems in comparison to soil-based culture (Chow et al., 2017).

Issues	Hydroponic system	Soil culture
Water	Efficient water usage; Irrigation water can be recycled or reused; No nutrient waste due to water runoff; Irrigation water is supplied directly to root areas; Possibility of controlling water holding ability by using different kinds of medium.	Insufficient water usage; Irrigation water cannot be recycled or reused; Eutrophication of the environment due to surface run-off Difficulties of the control of water-holding capacity.
Land usage and effect of environment	Less affected by soil and external factors; İndoor system and ease of nutrient control; Excellent control of environment temperature, humidity and lighting time.	Limited by different soil types; Subjected to the changing external environment.
Fertilizers and nutrient solution	Even distribution; Efficient use of fertilizers and cost saving; Ease of pH control.	Uneven distribution; Excessive use of fertilizers; Variation of pH with the changing weather and external factors.
Quantity and quality of crop	Stable and even amount of crop production.	Unstable crop production and subjected to pests/soilborne pathogens.

2.1.2. Benefits of aquaponic systems

Aquaponics is an atypical and complex food production technology due to its integrative nature and diverse application scenarios from high-tech to low-tech. The complexity of a system and its application in different environments potentially impacts the realization of benefits in all aspects: economic, environmental, and social.(Rizal et al. 2018a)

2.1.2.1. Economic benefits

Today, crop cultivation and fish farming occupy a large part of the earth's surface and have a strong negative impact on the environment by causing soil erosion, soil and groundwater contamination by pesticides, fertilizers, and animal waste, greenhouse gas production, and in many other ways. Combining crop production and fish farming in a closed

aquaponics system significantly reduces environmental impact (Goddek et al. 2019). Aquaponics systems operate virtually waste-free and have no measurable impact on the soil unless new land is required to install aquaponics. Even if a relatively small amount of waste (in the form of sludge) is generated, it can be easily composted and converted into useful products (Rizal et al. 2018b). The feasibility of aquaponics on an industrial scale depends on the ability to achieve efficient, high-yield systems. Fish feed is the largest cost factor in intensive aquaculture. The environmental and economic benefits can be significantly improved by formulating alternative fish feeds and reducing fishmeal and fish oil in feeds. Additionally, contamination of feed with mycotoxins due to feed ingredients or poor storage conditions is often overlooked and is dangerous as it can cause many health problems to fish and reduce yield and economic benefits (Rizal et al., 2018).

2.1.2.2. Environmental benefits

Aquaponic systems stand as a beacon of sustainability in agriculture, embodying numerous environmental benefits. Their standout feature lies in water conservation achieved through an intricate closed-loop system, where water perpetually circulates between fish tanks and plant beds. This design significantly slashes water usage, a stark contrast to the susceptibility of traditional soil-based farming to water loss through runoff and evaporation. Their reduced reliance on external inputs, such as synthetic fertilizers and pesticides, not only lessens environmental pollution but also mitigates the peril of detrimental runoff seeping into nearby water bodies (Rizal et al. 2018b).

What sets aquaponics apart is their potential for low energy consumption, especially when integrated with renewable energy sources. This starkly contrasts with the energy-intensive nature of conventional farming practices, like mechanized plowing and irrigation. Furthermore, their adaptability across diverse settings, including urban spaces and underutilized areas, showcases their prowess in promoting efficient land utilization, an exemplar seen in vertical or rooftop aquaponic setups flourishing even in densely populated regions (Rizal et al. 2018a).

These systems orchestrate a harmonious ecosystem where fish waste becomes nourishment for plants, reciprocated by the plants purifying water for the fish (**Figure 3**). This symbiotic relationship not only fosters biodiversity within the system but also acts as a shield against soil erosion and degradation, in direct contrast to the common soil loss and reduced fertility seen in traditional farming methods. This holistic approach to agriculture not only

redefines sustainable farming but also offers a tangible pathway toward preserving our environment for generations to come (Rizal et al. 2018a).

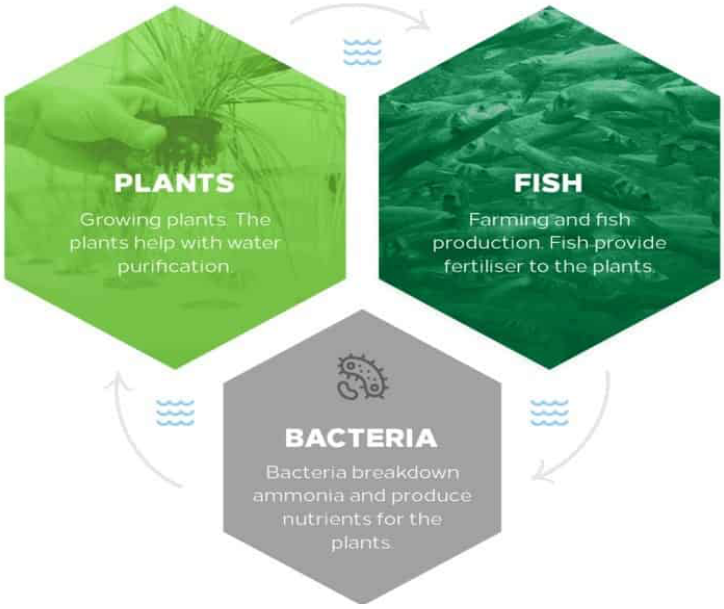


Figure 3. The environmental impact (Wei et al., 2019).

2.1.2.3 Social benefits

Aquaponics is already widely used in elementary and junior high school science education and vocational training. However, little has been done to assess social aspects (health, well-being, education and learning from demonstration projects). There are still technical and pedagogical issues that need to be overcome before aquaponics units can be claimed to facilitate practical education. Another social aspect with great potential is community cohesion. However, the structure of such a system is different from that of a commercial city or industrial production, and therefore the evaluation of benefits will also be different. There may be trade-offs between technology and knowledge inputs (high-tech vs. low-tech) and the potential for social impact (Rizal et al., 2018).

2.1.3. Decoupled aquaponic systems

The classic aquaponic system, commonly referred to as the combined or one-loop aquaponic system, was described more than 30 years ago. Here, the aquaculture and hydroponic units are arranged in a single circuit, with treated water being sent from the aquaculture unit to the hydroponic unit and back. Inevitably, such systems provide the same water quality for both fish and plants, which leads to compromises in rearing conditions for each production line (Goddek et al. 2019). Perhaps because there is little commercial

application and most aquaponics systems are small units, the need for compromise and lack of production control are the main obstacles (Monsees, Kloas, and Wuertz 2017). Current efforts are aimed at separation systems arranged in separate circuits, where process water is primarily recirculated within each unit to better control species-specific requirements. Water is recirculated within each unit (recirculating aquaculture system or hydroponics) and water loss due to plant evapotranspiration is transferred from the aquarium to the hydroponic reservoir via a one-way valve as needed (Monsees, Kloas, et Wuertz 2017). Supplemented by channeling process water. This means that the water from the hydroponic unit is not drained into the aquarium and the conditions within the hydroponic unit can be managed independently if desired. To further improve water efficiency, the production greenhouse is equipped with an additional air conditioning system with an integrated cold trap to condense the evaporated water and condensate water (pure water) from the plants and recirculating aquaculture system into the circulation circuit It is listed in. Repurpose the aquaculture systems department (Monsees et al., 2017).

The decoupled aquaponic (**Figure *4***) system becomes advantageous since dissociated designs allow greater flexibility to optimize the water pH in the fish and plant components separately. Another benefit of decoupled systems arises from the possibility of making precise adjustments to nutrient concentrations to adapt to the specific needs of the system. This adaptation involves the intensive application of nutritional supplements in the cultivation water, especially after the fish rearing phase, aiming to promote plant growth (Lennard & Goddek, 2019).

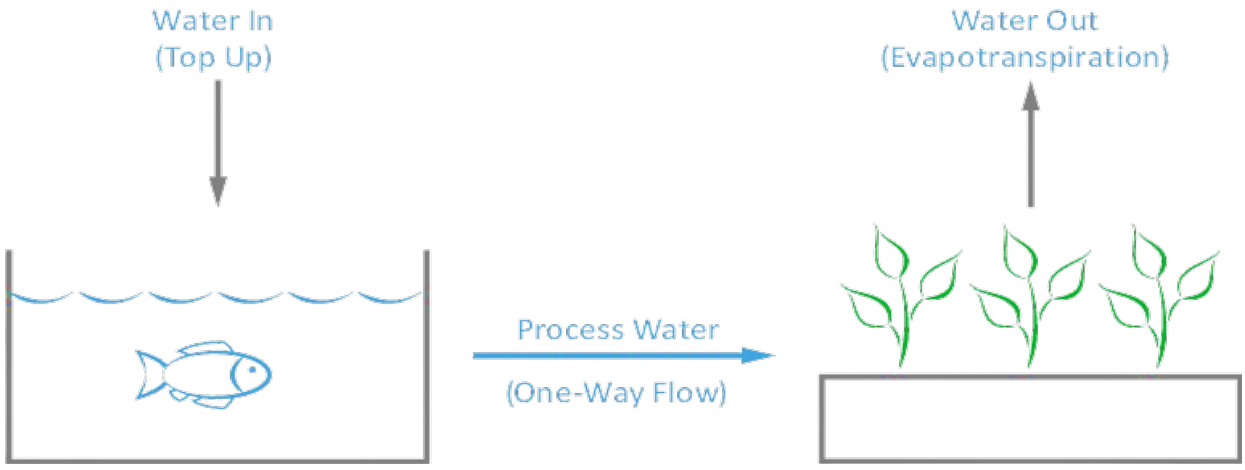


Figure 4. Simplified scheme of the main water flows within a decoupled aquaponic system (Goddek et al., 2019).

2.2. Brassicas

The Brassicaceae family includes many economically important species, especially edible oil plants, vegetables, spice plants, and forage plants. Cruciferous vegetables are highly nutritious and high in fiber. In addition, cruciferous vegetables contain a variety of bioactive chemicals, including glucosinolates and sulphur-containing that shows cancer-protecting. These beneficial anticancer effects of Brassicas vegetables are commonly associated in the literature with glucosinolates (GLSs), and some secondary metabolites in their composition, as well as other phenolic compounds, seed oils, and fiber (Ağagündüz et al., 2022). The common name, botanical classification and nutritional composition of the most usual Brassicas vegetables are shown in the **Table 2**.

Table 2. Common name, botanical classification, and nutritive composition of Brassicas vegetables (Ağagündüz et al., 2022).

Common name	Botanical classification	Nutritional composition (g/100g fresh weight - fw)					
		Water	Protein	Fat	Carbohydrate	Fiber	Energy (kcal/100 g fw)
Broccoli	*Brassica oleracea* var. *italica*	90	2.57	0.34	6.27	2.4	39
Cabbage	*Brassica oleracea* var. *capitata*	92.2	1.28	0.1	5.8	2.5	25
Cauliflower	*Brassica oleracea* var. *botrytis*	92.1	1.92	0.28	4.97	2	25
Rocket	*Eruca sativa* L.	91.7	2.58	0.66	3.65	1.6	25
Turnips	*Brassica rapa* subsp. *rapa*	91.9	0.9	0.1	6.43	1.8	28
Watercress	*Nasturtium officinale* L.	95.1	2.3	0.1	1.29	0.5	11
Radishes	*Raphanus sativus* L.	95.3	0.68	0.1	3.4	1.6	16

2.2.1. Rocket (Eruca sativa L.)

Rocket (also known as arugula and rucola, **Figure 5**) is a leafy vegetable that has become increasingly popular around the world, especially over the past 15 years. Two main species are grown primarily as salad crops, namely, *Eruca sativa* or *Eruca vesicaria* subsp. *sativa* (cultivated rocket), and *Diplotaxis tenuifolia* (wild rocket). Both species share a very distinctive peppery taste and aroma (Bell & Wagstaff, 2014).

Rocket is an endemic species of the Brassicaceae family which is produced mostly in Mediterranean countries such as Italy, Greece and Turkey. It is a dark green annual plant, about 20 to 50 cm in height, with a spicy-pungent taste. Since ancient times, the rocket plant has been a source of nutrition, an herb, an aphrodisiac, and a medical plant, and has other uses. According to data from 2010, the annual production of rocket in Turkey was 4058 tonnes, but because most production is carried out by amateur gardeners or on small plots, it

is difficult to know how much is produced. In addition, it has been extensively reported in the literature that rocket has astringent, diuretic, digestive, emollient, depurative, laxative, rubefacient, tonic, stomachic, anti-inflammatory for colitis and stimulant properties. In the cosmetics sector, it has uses such as, for promoting hair regrowth, the treatment of oily scalp, and as a facial tonic. With all these qualities, it is also an easy plant to grow and has often been chosen as experimental material by researchers (Barlas et al., 2011; Bell & Wagstaff, 2014).

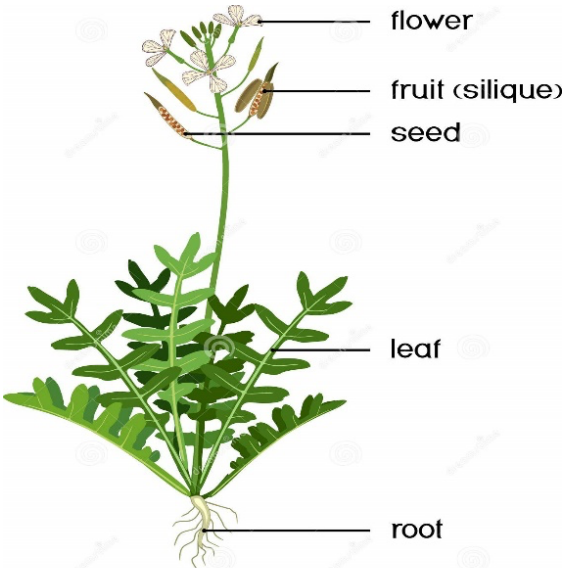


Figure 5. Morphology of rocket plant with green leaves (Barlas et al., 2011).

2.2.2. Rocket nutritional value

Rocket is renowned for its high nutritional value. These leafy greens are packed with a variety of beneficial compounds, such as vitamin C, beta-carotene, and lutein, which help protect the body's cells from oxidative stress and inflammation. These antioxidants support overall health by reducing the risk of chronic diseases and boosting the immune system (Cavaiuolo et Ferrante 2014). In **Table 3** is described the major compounds found in rocket and the corresponding function/effect to the consumer.

Glucosinolates, sulphur-containing compounds, are also found abundantly in rocket, contributing to the distinctive peppery flavour. These compounds have been extensively studied for their potential anti-cancer properties. When consumed, glucosinolates break down into biologically active compounds like isothiocyanates and indoles, which have been shown to inhibit the growth of cancer cells and protect against DNA damage (Cavaiuolo et Ferrante 2014).

Table 3. Chemical Profile of Rocket per 100 grams (Cavaiuolo et Ferrante 2014).

Component	Type	Function/Benefit	Approximate Amount per 100g
Vitamins			
Vitamin A	Antioxidant	Vision, immune function, skin health	2373 IU
Vitamin C	Antioxidant	Immune support, collagen synthesis	15 mg
Folate (Vitamin B9)	B Vitamin	DNA synthesis, red blood cell formation	Not specified
Vitamin K	Blood clotting	Blood clotting, bone health	109 µg
Minerals			
Calcium	Mineral	Bone health, muscle function	160 mg
Potassium	Mineral	Heart function, fluid balance	369 mg
Magnesium	Mineral	Biochemical reactions, muscle and nerve function	Not specified
Phytochemicals			
Glucosinolates	Sulfur compound	Anti-cancer properties	Not specified
Flavonoids	Antioxidant	Anti-inflammatory effects	Not specified
Antioxidants			
Beta-carotene	Antioxidant	Precursor to vitamin A	Included in Vitamin A
Lutein	Antioxidant	Eye health	Not specified
Zeaxanthin	Antioxidant	Eye health	Not specified
Nitrates	Nitrate	Cardiovascular health, blood pressure regulation	Not specified
Iron	Mineral	Oxygen transport in blood	1.5 mg
Macronutrients			
Calories	Energy	Daily energy intake	25 kcal
Fiber	Carbohydrate	Digestion, gut health	1.6 g
Protein	Macronutrient	Muscle repair and growth	2.6 g
Fat	Macronutrient	Energy storage, cell structure	0.7 g
Carbohydrates	Macronutrient	Energy source	3.7 g

Rocket is a hyper-accumulator of nitrates. Dietary nitrates can be beneficial, particularly for cardiovascular health, since help improve blood flow and reduce blood pressure by being converted into nitric oxide in the body (Cavaiuolo et Ferrante 2014). On the other hand, excessive nitrate intake has been linked to health risks, including the potential for gastrointestinal cancer. This risk arises from the formation of nitrosamines, compounds that can form when nitrates interact with proteins during digestion (Cavaiuolo et Ferrante 2014).

The high nutritional value of rocket leafy salads makes them a healthy addition to the diet, providing essential vitamins and minerals while offering protective benefits against chronic diseases. However, due to their high nitrate content, it is advisable to consume them in moderation. This is particularly important for individuals who may be at higher risk of

nitrate-related health issues, such as those with gastrointestinal conditions (Cavaiuolo et Ferrante 2014).

2.2.3. Sustainable production of rocket

2.2.3.1. Production of rocket through hydroponics

Research shows that rocket can achieve high yields in hydroponic systems due to optimal nutrient availability and controlled environmental conditions (Barbosa et al. 2015). Studies report that hydroponically grown rocket often outperforms soil-grown counterparts in terms of biomass production and harvest frequency (Kar et Kumar 2009). Rocket exhibits faster growth rates in hydroponic systems compared to soil, with controlled studies demonstrating reduced growth cycles, with some varieties reaching harvestable size in as little as 4 weeks.

Hydroponic systems allow precise control over nutrient delivery, leading to efficient nutrient uptake by rocket plants (Barbosa et al. 2015). This results in reduced fertilizer use and minimal nutrient runoff, making hydroponics an environmentally friendly option. Research has explored various nutrient formulations to optimize rocket growth, suggesting that a higher nitrogen concentration benefits leafy green growth, contributing to increased biomass and leaf size. Hydroponically grown rocket often has better quality attributes, including higher leaf turgor, consistent leaf size, and enhanced visual appeal (Kar et Kumar 2009).

Controlled conditions reduce the likelihood of pest and disease damage, further improving the quality. Some studies indicate that hydroponic cultivation can enhance the flavor profile of rocket, with the controlled environment and optimal nutrient supply leading to higher concentrations of flavor compounds (Barbosa et al. 2015). Hydroponic systems use significantly less water compared to traditional soil-based agriculture, which is particularly advantageous in regions facing water scarcity. Vertical farming techniques and stacking hydroponic setups allow for efficient space utilization, making it possible to produce large quantities of rocket in limited spaces. Despite higher initial setup costs, hydroponic systems can be economically viable in the long run due to higher yields, faster crop cycles, and reduced input costs(Kar et Kumar 2009). The ability to produce rocket year-round in controlled environments ensures a consistent supply, potentially leading to stable market prices and reduced dependency on seasonal variations (Barbosa et al. 2015).

2.2.3.2. Production of rocket through aquaponics

Rocket plants can achieve high productivity in aquaponic systems due to the nutrient-rich water from fish waste, which promotes healthy plant growth and often results in yields that match or exceed those of hydroponically grown rocket (Graber et Junge 2009). Rocket grows robustly in aquaponics, reaching harvestable size in 4-6 weeks, thanks to the continuous supply of nutrients from fish waste (Goddek et al. 2019).

This natural fertilization process provides a balanced nutrient profile, leading to efficient nutrient uptake and minimal need for external fertilizers. The controlled environment in aquaponics enhances the quality of rocket, producing larger, better-textured, and more colorful leaves with improved flavor (Yep et Zheng 2019). Aquaponics is also environmentally sustainable, using significantly less water through recirculation and reducing waste by repurposing fish waste as plant nutrients. Economically, the dual production of fish and plants can be viable, potentially doubling revenue streams. Despite high initial setup costs, long-term savings on water and fertilizers, combined with revenue from both fish and plant sales, make aquaponics a feasible option (Graber et Junge 2009).

2.3. Fish species commonly used in aquaponic system

2.3.1. The Importance of Choosing the Best Fish for the Aquaponics System

Selecting the most suitable fish for the aquaponic system is a crucial decision that significantly influences the overall health and productivity of the aquaponic setup. Different fish species come with distinct preferences regarding water temperatures, diets, growth rates, and disease resistance. Opting for the wrong fish may disrupt the system's balance, impede plant growth, and pose risks to the fish population. On the contrary, choosing the appropriate fish can optimize nutrient production, contributing to the overall sustainability and efficiency of the aquaponics system (Gosh & Chowdhury, 2019).

One of the primary factors to weigh when choosing fish for aquaponics system is their temperature preferences. Fish can generally be classified into warmwater and cold-water species. Warmwater fish, such as tilapia, thrive in temperatures ranging from 75 to 85°F (24 to 29°C). In contrast, cold water species like trout flourish in cooler temperatures, typically between 50 to 70°F (10 to 21°C). The decision between warm water and cold-water species predominantly hinges on the climate and capacity to control temperatures within aquaponics setup (Gosh & Chowdhury, 2019).

Other factors to consider regarding the fishes are the balancing diet, fish population and size of aquaculture tanks. Maintaining a harmonious aquaponics system involves finding an equilibrium between the fish species, their quantity, and the nutritional requirements of crops. Overfeeding fish can result in excessive waste, whereas underfeeding may lead to nutrient deficiencies in your plants. Regularly monitoring and adjusting feeding practices are crucial for sustaining a well-balanced aquaponics system (Goddek et al., 2015). The size of the fish tank should be proportional to the size of plant beds. Fish produce waste continuously, and it's essential to ensure that there is enough water volume to dilute and distribute these nutrients effectively to the plants, while overcrowding can lead to stress, disease, and poor water quality for the fish. Calculating the appropriate stocking density based on the species and the tank size is essential (Knaus & Palm, 2017). Avoid overcrowding, as it can harm both fish and plants. Goddek et al. (2019) studied types of fishes and physical parameters to stablish one aquaponic system as described in **Table 4**.

Table 4. Types of fishes and physical parameters to stablish one aquaponic system (Goddek et al., 2019).

Fish species	**pH range**	**Temperature (**°C)	**Fish tank size (L)**
Tilapia	6.5-9	24-30	11-19
Trout	6.5-8	14-16	760
Catfish	7-8	18-32	at least 30
Calandino	6.5-8	15-20	75-113
Largemouth Bass	6-8	18-26	380 to 570
Salmon	7-8	13-18	1000
Koi	6.5-8	18-25	3800

2.3.2. Calandino (Squalius alburnoides)

Squalius alburnoides, commonly referred to as the Iberian chub or calandino (**Figure 6**), is a freshwater fish species endemic to the Iberian Peninsula, encompassing Portugal and Spain. Exhibiting a streamlined body with varying shades of silver or olive-green along its sides, a white belly, and distinct dark fins, this small to medium-sized fish thrives in rivers and streams characterized by clear to moderately turbid waters figure 6. Its habitat includes diverse aquatic environments such as pools, riffles, and runs. Noteworthy for its reproductive versatility, *Squalius alburnoides* engages in both sexual and unisexual reproduction, with females capable of producing offspring without male fertilization through a process known as

gynogenesis. Despite its adaptability, the Iberian chub faces conservation challenges in some regions, including habitat degradation, pollution, and the potential introduction of non-native species. Conservation efforts are essential to safeguard the integrity of local populations and ensure the continued ecological balance of these aquatic ecosystems (Collares-Pereira et al., 2013).

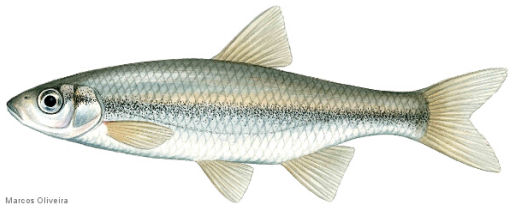


Figure 6. *Squalius alburnoides* (Crespo-López et al., 2006).

Most individuals of this species are hybrids that have different ploids - diploids (2n=50), triploids (3n=75) and tetraploids (4n=100) that are not morphologically distinguishable. However, triploid individuals are those that reach larger dimensions (around 13 cm) when compared to diploid individuals and tetraploids that rarely exceed 9 cm in length. The body of the *calandino* is narrower in diploids and tetraploids than in triploids (Crespo-López et al., 2006).

2.3.3. Ecological importance of aquaponic systems

Aquaponic systems integrate aquaculture and hydroponics into a closed-loop system where fish waste provides nutrients for plant growth, significantly reducing water use and nutrient losses compared to traditional agriculture. Studies suggest aquaponics can cut water consumption by up to 90%, making it sustainable in water-scarce regions (Goddek et al. 2019). The symbiotic relationship between fish and plants enhances nutrient cycling, converting fish waste into essential nutrients like nitrates and nitrites, thereby reducing the need for synthetic fertilizers and mitigating nutrient runoff (Graber et Junge 2009). By operating in controlled environments, aquaponics minimizes habitat destruction and biodiversity loss associated with traditional farming, contributing to conservation efforts (Goddek et al. 2019). These systems also offer climate resilience, enabling year-round crop production despite climate variability, ensuring food security in vulnerable regions (Yep et Zheng 2019). Additionally, aquaponics demonstrates energy efficiency by optimizing greenhouse conditions, which lowers energy consumption and greenhouse gas emissions compared to conventional agriculture (Goddek et al. 2019).

3. Framework and objectives

3.1. Main goal

This study investigates the effects of wastewater from calandino fish (*Squalius alburnoides*) production on the growth and quality of rocket plants (*Eruca vesicaria* L.) using a decoupled aquaponics system, with the aim to comprehensively evaluate the multifaceted aspects of an aquaponics system, focusing on analysing its diverse components.

3.2. Specific objectives

The specific objectives are described below:

- Assess the growth performance and physical condition of fish raised in the aquaculture system;
- Evaluate the physicochemical aspects of the wastewater from the fish production, regarding pH, electrical conductivity, ammonia nitrate, total nitrogen, and phosphorus concentrations;
- Assess microbiological quality of wastewater by quantifying colony counts at 22°C and 36°C, total coliforms, Escherichia coli, Clostridium perfringens, and intestinal enterococci;
- Post-harvest analysis of the phytosanitary conditions and the overall yield of the rocket crop, including leaf damage, fresh weight, leaf number, and leaf area;
- Nutritional evaluation of rocket by AOAC methods, namely, moisture, ash, total fat, crude protein, carbohydrates, and energy value;
- Determination of the chemical composition in:
    - Sugars by HPLC coupled to a Refractive Index (RI) detector;
    - Organic acids by UFLC coupled to a Diode Array Detector (DAD);
    - Fatty acids by Gas Chromatography (GC) coupled to a Flame Ionization Detector (FID);
- Comparison between the commercially and aquaponically grown rocket regarding nutritional profile and chemical composition.

4. Methodology

The decoupled aquaponics system, independent aquaculture and hydroponics systems, was installed in the Aquaculture Laboratory and in the Greenhouses and Nurseries facilities, respectively, of the Escola Superior Agrária, Instituto Politécnico de Bragança, Portugal, for a period of seven months, between April 2024 and October 2024.

4.1. Reagents and samples

The distilled water used was treated in a MilliQ purification system (Millipore, model A10, Billerica, MA, USA). The rocket (*Erucara visicaria* var. sativa) seeds **(**flora Lusitana Lda., Cantanhede, Portugal) used for germination and growing in the hydroponics system were obtained from a local retailer in Bragança, Portugal. Samples of vegetative parts of commercial rocket (Vitacress, Odemira, Portugal) were also obtained from a local retailer. These commercial samples were weighed, frozen and freeze-dried to obtain a fine, dry powder (20 mesh), which was stored under controlled humidity and light conditions for later analysis of its nutritional profile and chemical composition.

The standard reference mixture of fatty acid methyl ester (FAME) 37 (standard 47885-U) was purchased from Sigma-Aldrich (St. Louis, MO, USA), as well as the isomers of individual fatty acids, sugars (D(+)-saccharose and D(+)-melezitose) and organic acid standards (oxalic acid, shikimic acid, fumaric acid, and quinic acid). For tocopherol analysis, racemic tocol (50 mg/ml) was purchased from Matreya (Chalfont, PA, USA). The HPLC grade solvents n-hexane (95%) and ethyl acetate (99.8%) were purchased from Lab-Scan (Lisbon, Portugal).For analysing phenolic compounds, acetonitrile (99.99%, HPLC grade) and formic acid were purchased from Fisher Scientific (Lisbon, Portugal) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

4.2. Aquaculture system

The tank system in the Aquaculture Laboratory (Aquaneering Systems ©) consisted of 5 rows, each containing 8 PETG (Polyethylene Terephthalate Glycol) tanks, a transparent, durable, and non-toxic thermoplastic with a 16 L capacity. In this closed system, there are four filtration stages that provide clean, recycled water for the 40 tanks (Burg et al. 2014).

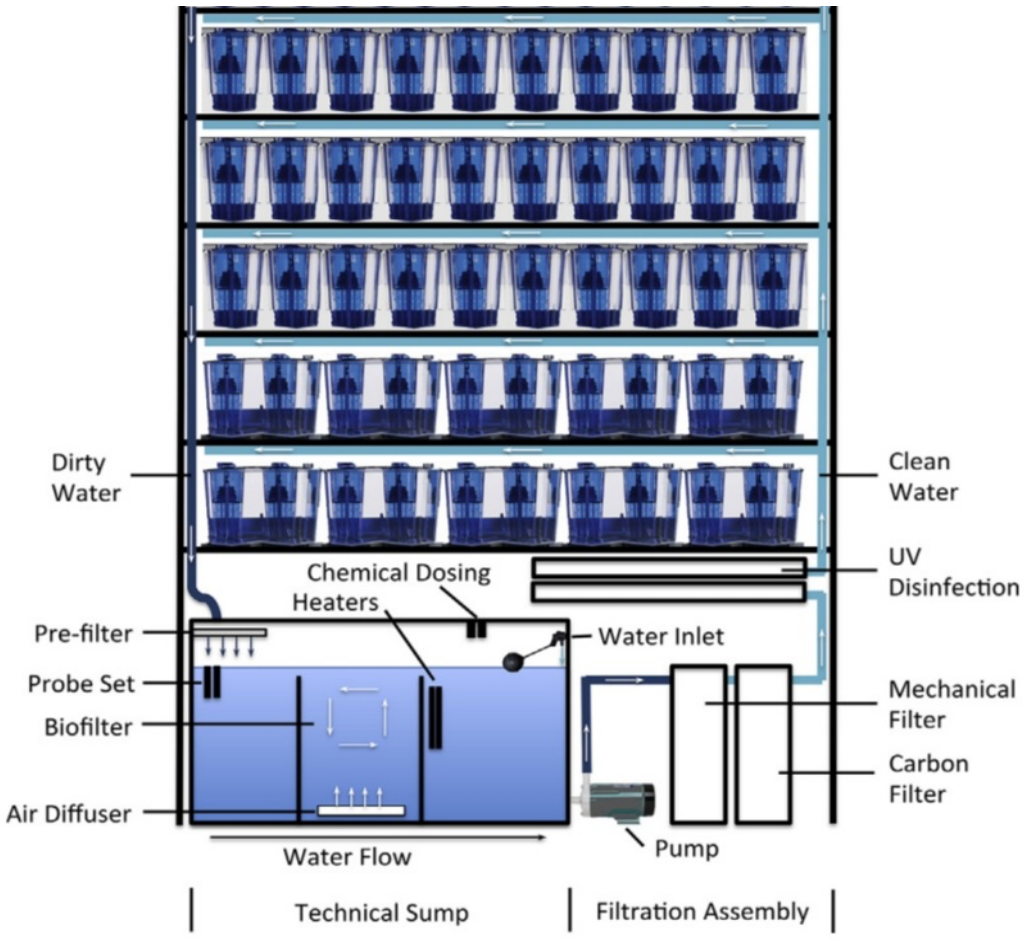


Figure 7. Description of the Aquaneering systems adapted in the work (Cockington 2020).

The Aquaneering system operates through four distinct sections: i) **First section** – Mechanical filtration, where water is forced through a filter that retains solid waste particles. The filter was replaced frequently due to significant debris accumulation. ii) **Second section** – After mechanical filtration, the water is pumped into a fluidized bed biological filter (FBB). At this stage, water is forced to the bottom of the FBB, creating agitation between the filter medium and the water. This movement prevents system clogging and maximizes surface area for the growth of nitrifying bacteria. These bacteria convert harmful substances, such as ammonia and nitrites, into less harmful nitrates, ensuring water stability and quality without the need for frequent maintenance, unlike other filtration methods (Burg et al. 2014). iii) **Third section** – After passing through the FBB, the water flows back into the reservoir chamber, where it is purified by two activated carbon filters and then directed to the supply lines of the rack system containing the 40 tanks. iv) **Fourth section** – Before entering the tanks, the water passes through a 40-watt UV light reactor equipped with a sterilizing lamp inside a quartz sleeve. This process provides final water disinfection, achieving a minimum of

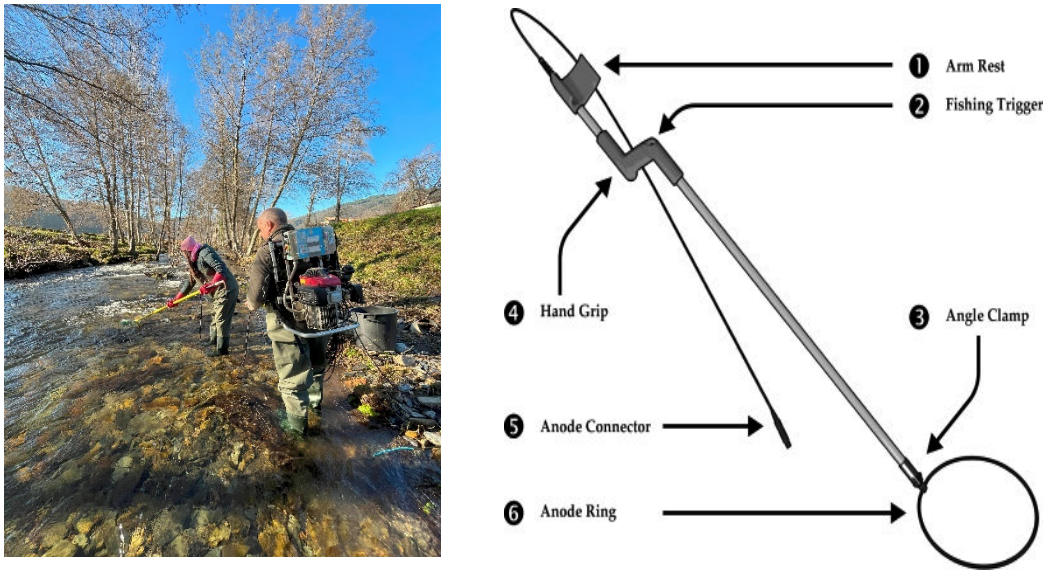
90.000 µwatts.s/cm², to eliminate bacteria and microorganisms. This is the final step before the water is routed to the tanks, repeating the process in a closed system, which can renew up to 10% of the circulating water.

The system is also equipped with an electronic Control Box and an Aqualogic Chiller, allowing constant control and maintenance of the pre-set water temperature (Cockington 2020).

During the seven months of system operation, the following physico-chemical water parameters were monitored weekly: temperature, pH, and conductivity (Milwaukee Instruments, USA), oxygen concentration, and saturation (Hach, Portugal). Tanks and filters cleaning procedures were conducted at least once a week.

4.3. Fish species capture

The capture of wild specimens of the fish species selected for this study, *Squalius alburnoides*, commonly known as "calandino" followed the methodology described in the Manual for the Biological Assessment of Water Quality in River Systems according to the Water Framework Directive – Sampling and Analysis Protocol for Fish Fauna (INAG, 2008). According to the protocol, the specimens were captured using (Hans Grassl™ ELT60II-GI; 300-600 V, DC, 2200W). Electrofishing is a direct and efficient sampling method that results in near-zero mortality rates (**Figure 8**) (Snyder, 2003).

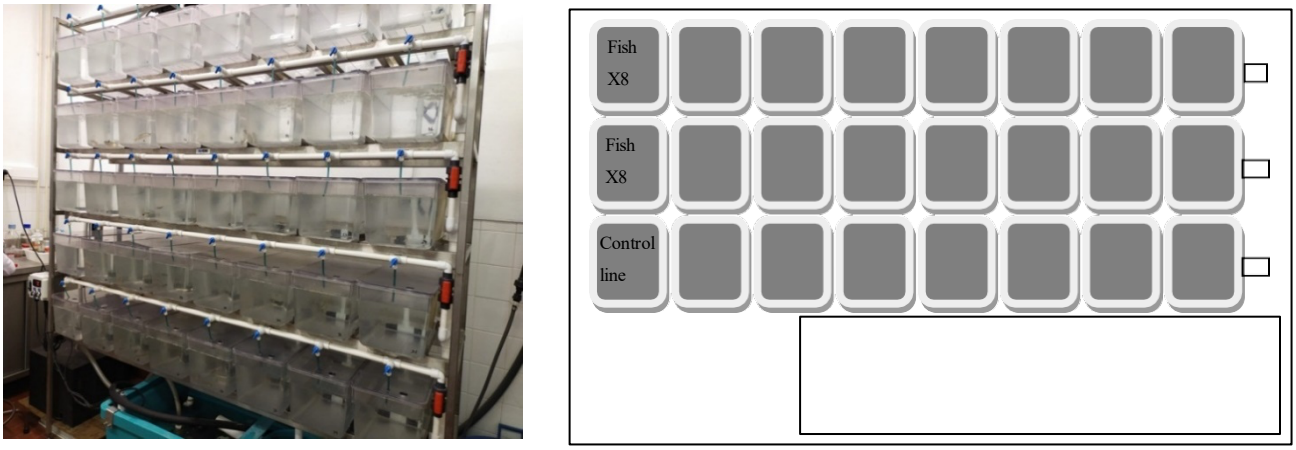


A B

Figure 8. Electric fishing: sampling of fish on the Sabor River (spring season 2024) (A) and electrofishing backpack anode system (Nielsen, 1998) (B).

Fish processing included careful handling and *in situ* identification down to the species level. Biometric data were also obtained for the selected fish, with total length measured using an ichthyometer (precision of 0.1 cm) and biomass determined using a portable balance (precision of 0.1 g, Kern, Portugal).

A total of 200 *S. alburnoides* specimens, originated from the Sabor River, of similar size (Mean ± SD, cm), were selected and distributed across the available tanks (total number of tanks: 24). The collected specimens were transferred to the Aquaneering Systems© individual fish housing system, as described above. It was decided to keep the 1st line empty, the 2nd line with fish for replacement in case of mortality, while the experiment was carried out on the last 3 lines of the system, as follows: 3rd line with 8 fish/tank, 4th line with 8 fish/tank and the 5th line without fish (control line) as shown **Figure 9**. The initial acclimatization period of the fish to captivity and artificial feed adaptation lasted 15 days, after which the experiment begins. Fish were fed daily, receiving a feed portion equivalent to 3% of their body weight, as described by Boxman et al. (2018). In the event of a fish's death, it was replaced by another specimen of similar weight and size.



A **B**

Figure 9. Actual implementation (A) of the aquaponics system; One-factor experimental design (B).

4.3.1. Fish growth assessment

To evaluate fish growth, biometric data on the length and weight of all fish specimens under study were collected. To minimize animal stress, measurements were taken only at the beginning and end of the experiment. The length-weight correlation was calculated using **Equation 1** (Le Cren, 1951; Froese, 2006):

Equation 1. Length-weight relationship

$$W = a * L^b$$

Where:

- **W**: Fish weight in grams (g);
- **L**: Total fish length in millimetres (mm);
- **a** and **b**: equation coefficients, where **b = 3** indicates isometric growth, and **b ≠ 3** indicates allometric growth.

The physical condition of the fish was determined using Fulton's Condition Factor (K), as shown in **Equation 2** (Ricker, 1975):

Equation 2. Fulton Condition Factor (K Factor).

$$K = \frac{(100 * W)}{L^b}$$

Where:

- **K**: Condition factor or physical condition coefficient;
- **W**: Individual fish weight in grams (g);
- **L**: Total fish length in centimetres (cm);
- **b**: Length-weight equation coefficient.

4.4. Quality of the wastewater fish

During the experimental period (7 months), chemical and microbiological analyses were performed on wastewater resulting from fish production in the aquaculture system tanks. Sampling for both analyses was conducted in only 3 tanks per row.

4.4.1. Chemical analyses of wastewater

The chemical analyses (nitrates, nitrites, phosphates and oxygen levels) were assessed every two weeks during the experimental period, with water samples collected for irrigation (described below) and for analysis at the same time.

4.4.1.1. Nitrate Content

The determination of nitrate content was performed according to the procedures described by Rodier (1981), with some adaptations. Initially, 1 mL of each collected water sample was combined in a glass mortar, followed by the addition of 4 drops of 0.1 M NaOH

and 1 mL of 5% sodium salicylate. After careful homogenization, the samples, including a blank prepared using only distilled water, were subjected to total evaporation until a white residue formed. Then, 1 mL of concentrated H2SOa was added to the samples, allowing them to cool completely. Afterward, 10 mL of distilled water and 10 mL of $NO3^{-}$ were added. Nitrate content was determined by spectrophotometry at 415 nm using quartz cuvettes, and results were expressed in mg/L.

4.4.1.2. Nitrite Content

For nitrite content analysis, 10 mL of each water sample was placed in Falcon tubes, followed by the addition of 0.4 mL of Zambelli reagent (Sigma-Aldrich, St. Louis, USA). A blank sample (distilled water) was also prepared using the same procedure. After homogenizing the samples, they were kept in the dark for 10 minutes. Subsequently, 0.4 mL of concentrated NH3 was added to the samples. Nitrite content was determined by spectrophotometry at 435 nm using acrylic cuvettes, and the results were expressed in mg/L.

4.4.1.3. Phosphate Content

Phosphate content determination was performed according to Tolgyessy (1993). Initially, 10 mL of each water sample was placed in Falcon tubes, followed by the addition of 0.2 mL of H2SOa (11 N), 0.4 mL of 5% ascorbic acid, and 0.8 mL of ammonium molybdate solution (40 g/L). A blank sample (distilled water) was also prepared using the same procedure. After homogenization and a 30-minute dark incubation, the samples were analysed by spectrophotometry at 800 nm using acrylic cuvettes, and results were expressed in mg/L.

4.4.1.4. Oxygen levels

The Winkler Method is a widely used technique for measuring dissolved oxygen levels in water, providing insight into water quality and aquatic health. The process starts by collecting a water sample with minimal air exposure to avoid altering the oxygen content. Manganese sulfate (MnSOa) and alkaline potassium iodide (KI) solutions are added as reagents, which react with the dissolved oxygen to form a brown manganese oxide precipitate. This is followed by the addition of sulfuric acid (H2SOa) to dissolve the precipitate and release iodine in proportion to the original oxygen content. The sample is then titrated with a sodium thiosulfate (Na2S2O3) solution until a clear endpoint is reached, indicating that all iodine has reacted. The amount of sodium thiosulfate used in the titration is directly related to the

concentration of dissolved oxygen in the water, providing a reliable measurement for evaluating water quality.

4.4.2. Microbiological analyses of wastewater

Microbiological analyses (total coliforms and total bacteria) were performed in the end of the experimental period. Water collection for microbiological analyses was carried out using previously autoclaved submersible glass sampling bottles.

4.4.2.1. Count of cultivable microorganisms

The colony count of microorganisms at 22 ºC and 37 ºC followed the protocol established by ISO 6222:1999 R(E). Initially, the necessary dilutions were prepared, where 1 mL of each collected water sample was transferred in duplicate to tubes containing 9 mL of peptone broth. After homogenization, 1 mL from each dilution was aseptically transferred to a new tube, repeating this process until the appropriate number of dilutions was achieved. Subsequently, 1 mL from each tube containing the final dilution was transferred to sterilized Petri dishes. Once the inoculum was evenly distributed across the plates, 15 to 20 mL of yeast extract agar medium (Himedia, India) was aseptically added. After carefully homogenizing the plates and solidifying the culture medium, the plates were incubated at 22 ºC and 37 ºC for 44 ± 4 hours. At the end of the incubation period, colonies were counted, and results were expressed in Colony Forming Units (CFU)/mL for each incubation temperature.

4.4.2.2. Analysis of Clostridium perfringens

The detection and quantification of *C. perfringens* spores were carried out according to the methodology described in ISO 14189:2013. Initially, 100 mL of water samples were transferred into sterilized tubes and heated in a water bath at 60 ± 5 ºC for 15 minutes (**Figure 10**). After cooling, using sterilized tweezers, a cellulose acetate filter membrane (0.2 µm pore size) was carefully placed in the filtration apparatus, and the 100 mL of solution was vacuum filtered. The filter membranes were removed and inverted onto sterile Petri dishes, to which 18 mL of TSC agar base (PanReac AppliChem ITW Reagents, Barcelona) was first added, followed by egg yolk emulsion (Himedia, India) and a 4% cycloserine solution (Acros Organics, China) to create anaerobic conditions before the medium solidified. The plates were then incubated at 37ºC for 20 ± 4 hours. After incubation, black-coloured colonies were counted, and the results were expressed in CFU/100 mL of the sample.

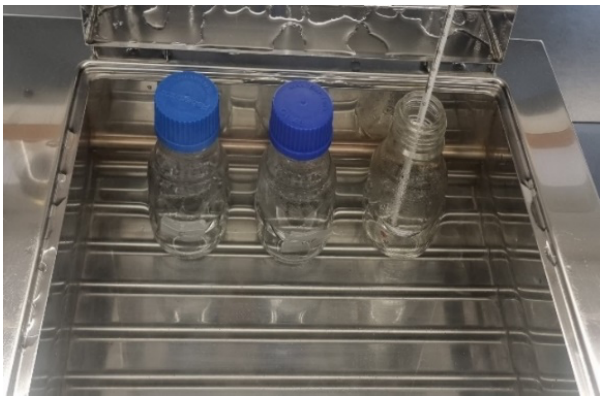


Figure 10. 100 mL of water samples heated in a water bath at 60 ± 5 ºC (15 minutes).

4.4.2.3. Analysis of intestinal Enterococci using the membrane filtration method

The detection and quantification of intestinal *Enterococci* followed the ISO 7899-2:2000 standard. First, 100 mL of the water sample was vacuum filtered through membrane filters with a 0.45 µm pore size. The filters were then carefully transferred to Petri dishes containing Slanetz-Bartley medium (Liofilchem, Italy) using sterile tweezers. After 48 hours of incubation at 37 ºC, colony formation was confirmed through Gram staining. Colonies were transferred onto a slide under aseptic conditions, followed by the application of hydrogen peroxide. A positive result was indicated by the absence of bubble formation, and a negative result by bubble formation. In positive cases, an additional test was performed where colonies were transferred to Petri dishes containing Bile Esculin Azide Agar (Himedia, India) and incubated at 44 ºC for 2 hours. The confirmation of intestinal *Enterococci* was based on the appearance of dark-colored colonies. Results were expressed in CFU/100 mL of the sample.

4.4.2.4. Analysis of total coliforms, thermotolerant coliforms, and Escherichia coli using the most probable number (MPN) method

The detection of total coliforms, thermotolerant coliforms, and *E. coli* followed the ISO 9308-2:1990 (E) protocol. For a presumptive test, 10 mL, 1 mL, and 0.1 mL of each water sample were distributed into distinct sets of tubes. The first set contained three tubes with 10 mL of double-concentrated lauryl sulfate tryptone (LST) broth; the second set contained three tubes with 9 mL of single-concentration LST broth; and the third set had three tubes with 9.9 mL of single-concentration LST broth. After homogenization and the addition of Durham fermentation tubes, the samples were incubated at 37ºC for 24 to 48 hours. Results were considered positive if gas formation was observed in the Durham tube, with those samples then being used in subsequent analysis stages.

4.4.2.5. Total coliform analysis

For the confirmation of the presence/absence of total coliforms, 0.1 mL from the positive tubes of the presumptive test was transferred to tubes containing 9.9 mL of "brilliant green lactose bile broth" (BGLB) (Biokar Diagnostics, Beauvais), then transferred to Durham tubes and incubated at 37ºC for 48 hours. The presence of gas formation in the Durham tube indicated a positive result for total coliforms. Results were obtained by combining the tubes with positive results, expressed in MPN/100 mL of the sample.

4.4.2.6. Analysis of thermotolerant (fecal) coliforms and E. coli

For the confirmation of the presence/absence of fecal coliforms and *E. coli*, 0.1 mL from the positive tubes of the presumptive test was transferred to tubes containing 9.9 mL of EC broth, including Durham tubes, and incubated at 44.05 ± 0.2 ºC for 24 to 48 hours. In tubes where gas bubble formation was observed, a positive test result was indicated. A 0.1 mL sample was then transferred into new tubes with tryptone broth and incubated for 24 ± 2 hours at 44ºC. After this period, 0.2–0.3 mL of Kovacs reagent (Liofilchem, Italy) was added, gently agitated, and allowed to rest. The presence of a red ring at the surface indicated a positive test for *E. coli*. Results were determined by combining the tubes with positive results, expressed in MPN/100 mL of the sample.

4.5. Hydroponic system

4.5.1. Rocket plant (Eruca sativa L.) germination

The germination process was carried out on a phenolic foam plate (GroHo Hydroponics, Lisbon, Portugal) with 92 cells, each measuring 2.0 x 2.0 x 2.4 cm. Phenolic foam is a sterile substrate made from phenolic resin, mainly used for plant germination and rooting. The foam was prepared according to the supplier's instructions. Specifically, the foam was fully submerged in tap water until saturated, after which the excess water was drained and the foam placed in a tray. Two to three rocket plant (*Eruca sativa* L.) seeds were inserted into each foam cell. Water was added again to the tray until the level reached about 1 cm in height, and the setup was kept in a dark place until the rocket seeds germinated (5 to 15 days). Epigeal seed germination was monitored daily, and after the seed coat ruptured with the emergence of the radicle (embryonic root), two cotyledons appeared, marking the stage when the seedlings were transferred, along with the phenolic foam (**Figure 11**), into the

perforated hydroponic channels. During the germination period, water was replenished in the tray whenever the level dropped below 1 cm.

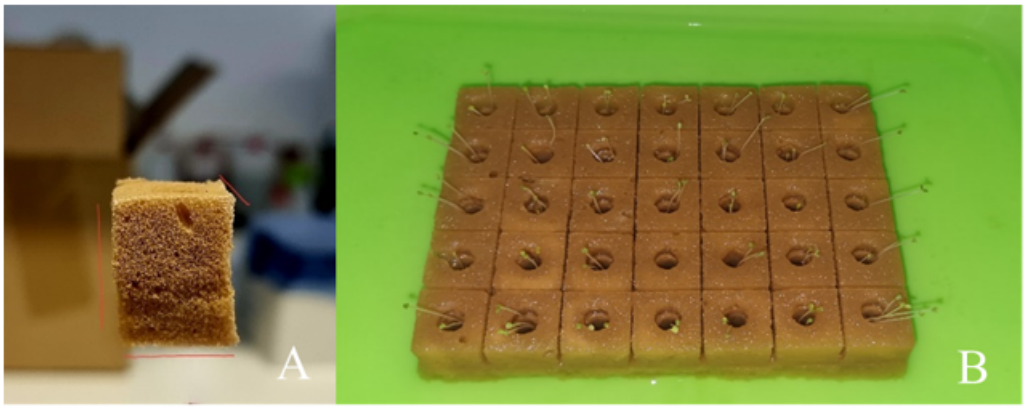


Figure 11. *P*henolic foam plate (2.0 x 2.0 x 2.4 cm) (A) *and* rocket plant germinating (5th day, B).

4.5.2. Experimental Design of the Hydroponic System in the greenhouse

For each of the 24 aquaculture tanks (section), a corresponding perforated hydroponic channel with four plugged holes (closed system, **Figure 12**) was installed. The perforated hydroponic profile (GroHo Hydroponics, Lisbon, Portugal) of 80 mm (bottom width: 7 cm; base width: 8 cm; height: 4.5 cm; and hole/plant diameter: 5.5 cm) is most suitable for plant production lines and is made from resinous material compliant with EC Regulation 1935/2044 of October 27, 2004.

Figure 12. Hydroponics profile with four buffered perforations.

Four germinated rocket plants (aged 5 to 15 days) were placed per hydroponic profile along with the phenolic foam, using mesh baskets as shown in **Figure 13** and in accordance with the one-factor experimental design described in **Figure 9**. During the 7 months experimental period, water from each tank was collected weekly and used to irrigate the corresponding hydroponic profile, with any remaining water from the profile being removed beforehand. As mentioned earlier, the following physical-chemical parameters of the tank

water were monitored weekly, prior to water collection: temperature, pH and conductivity (Milwaukee Instruments, USA). To facilitate the process, the water samples were collected in 300 mL bottles, with 250 mL used for irrigation and the rest for subsequent chemical analyses (previously described). To track the growth of the rocket plant, measurements of the plant's aerial part and leaf counts were taken every two weeks.

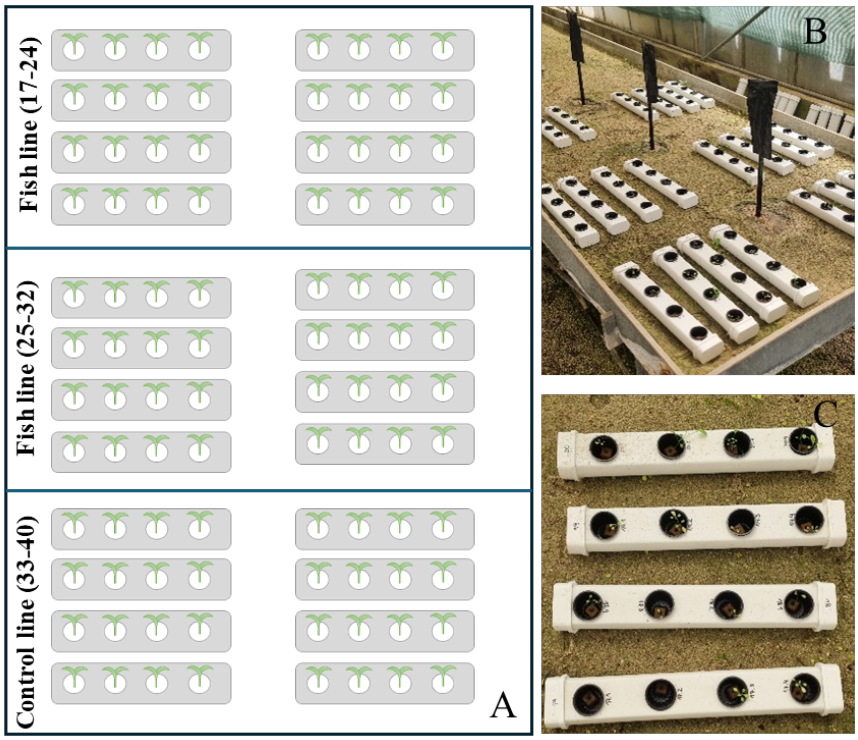


Figure 13. Description of the hydroponic system installation in the greenhouse (experimental design, A), implementation of the hydroponic system (B and C).

4.5.3. Experimental Design of deep-water culture (DWC) in small home hydroponic systems

The system was prepared with clean, dechlorinated water in the containers and an air pump was installed to ensure constant aeration. The hydroponic nutrient solution was then added, adjusting the electrical conductivity (EC) to around 1.5-2.0 mS/cm and the pH to 5.5-6.5 for optimum nutrient absorption. The germinated rocket plants were transplanted into net pots, ensuring that the roots hung down into the nutrient-rich water. The system was installed in a place with natural light.

4.5.3.1. Deep-water culture using wastewater from aquaculture system

The germinated rocket seedlings were planted in a deep-water hydroponic growing system utilising aquaculture water. The process commenced with the filling of the system's containers with water from an established aquaculture facility, which contained naturally occurring nutrients derived from fish waste. The water was certified to be free of contamination and debris. An air pump was connected to air stones to oxygenate the water, thereby ensuring adequate oxygenation of the plant roots. The pH of the water was adjusted to an optimal range of 5.5 to 6.5, as the aquaculture water exhibited fluctuating pH levels. The electrical conductivity (EC) was corrected to a range of 1.5 to 2.0 mS/cm, with the addition of a minimal quantity of hydroponic nutrient solution, if necessary, to replenish the nutrient levels for the rocket plants.

The germinated seedlings were meticulously positioned within net pots containing a supportive medium, such as clay pebbles or rock wool. This ensured that the roots extended downward into the nutrient-rich aquaculture water. The net pots were positioned on the system's lids in a manner that ensured the tops of the plants were above the surface, while the roots remained submerged. The system was situated in an area with an adequate supply of natural light or artificial illumination, in accordance with a photoperiod of 16 hours of light and 8 hours of darkness. The water quality was subject to regular monitoring, with parameters such as ammonia, nitrate, pH and EC being assessed to guarantee a balanced environment for the plants and the aquaculture water source (Mohanty et al. 2009).

4.5.3.2. Deep-water culture using solutions (A+B)

The germinated rocket seedlings were planted in a deep-water hydroponic growing system utilising distilled water with Solution A and Solution B. The system's containers were filled with distilled water. The nutrient solution was prepared by adding Solution A (which contains macronutrients such as nitrogen, phosphorus and potassium) and Solution B (which provides micronutrients such as iron, calcium and magnesium) to the water. The recommended dosage was used for each solution, with the aim of achieving an electrical conductivity (EC) of 1.5 to 2.0 mS/cm. The pH was adjusted to the optimal range of 5.5 to 6.5 for optimal nutrient absorption.

An air pump was connected to air stones in the containers to maintain adequate oxygen levels in the water, which is essential for the roots. The sprouted rocket seedlings were positioned in net pots containing a growing medium, such as clay pebbles or rock wool, ensuring that the roots were fully immersed in the nutrient-rich water. The cultivation

parameters (light, pH and EC) were comparable to those previously described for the aquaponics system. (Kar et Kumar 2009).

4.6. Nutritional profile of commercial rocket plants

4.6.1. Moisture

The moisture content was determined according to official analysis method nº 925.45b (AOAC, 2016), using an electronic moisture balance (ADAM, PMB 163, Oxford, USA), approximately 3 g of commercial rocket plant was weighed until total moisture removal by infrared radiation. The moisture percentage was calculated from the difference between the initial and final mass of the sample and expressed in g/100 g dry weight (dw).

4.6.2. Ash Content

Ash content was determined according to official analysis method nº 935.42 (AOAC, 2016). Initially, 0.250 g of rocket plant was placed in pre-calcined, weighed, and identified porcelain crucibles. The samples were then placed in a muffle furnace (IVYMEN, N-8L, Barcelona, Spain) at 550 ºC for 6 hours until white ash was obtained (complete calcination). The crucibles with calcined samples were cooled to room temperature (~25 ºC) in a desiccator and subsequently weighed until constant weight was achieved. The ash percentage was calculated from the difference between the initial and final sample mass and expressed in g/100 g dw.

4.6.3. Total Fat Content

The determination of total fat content in the rocket plant was performed according to official analysis method nº 989.05 (AOAC, 2016). Approximately 3 g of rocket sample was weighed into a paper cartridge placed in a Soxhlet-type fat extractor, using petroleum ether for extraction at approximately 120 ºC with a 6-hour cycle. The results were expressed in g/100 g dw, calculated by the gravimetric difference between the initial mass and the mass of fat obtained.

4.6.4. Crude Protein

The crude protein content was determined using the macro-Kjeldahl method following official analysis method nº 991.02 (AOAC, 2016), employing a conversion factor of 6.25 to

convert nitrogen content (N) into total protein, as suggested by Xu et al. (2019). Initially, 0.25 g of commercial rocket plant, two catalyst tablets (Kjeltabs), and 15 mL of concentrated sulfuric acid (H2SOa) were added to Kjeldahl tubes. The tubes were placed in a digestion block at 400 ºC for 70 minutes, followed by a cooling period, after which 25 mL of distilled water was added. Using the Kjeldahl analyzer (Velp Scientifica UDK 152), NaOH is added to the tubes containing the digested sample through back titration. This addition will release nitrogen in the form of NH3, which is then collected by steam distillation into a 0.1N H2SOa solution. Finally, a titration is performed with 0.1N NaOH, using methyl red as an indicator to calculate the amount of nitrogen. The nitrogen content is multiplied by a correction factor (N x 6.25), as shown in **Equation 3**, and the results are expressed in g/100g dw.

Equation 3. Calculation of crude protein content (g/100g dw).

$$\text{Protein}\left(\frac{\text{g}}{100\text{g}}\text{dw}\right) = \%\,\text{nitrogen (N)} \times \text{Conversion factor}$$

4.6.5. Total dietary fiber

The total dietary fiber content (soluble and insoluble) was determined by official analysis methods nº 991.43 and 992.16 (AOAC, 2016). In 50 mL Falcon tubes, previously wrapped in aluminum foil, 250 mg of sample were weighed and 12.5 mL of 0.08 M phosphate buffer at pH 6.0 were added plus 10 µL of a-amylase solution of *Bacillus licheniformis*. The tubes were then placed in a thermostated bath (Julabo, SW22; Seelbach, Germany) at 95 °C, with shaking at 5 min intervals. After 15 min of heating, they were removed and cooled until they reached room temperature (~25 ºC). Then the pH of the solution was adjusted to 7.5 ± 0.2 pH by adding 1 M sodium hydroxide solution, to which, immediately after adjustment, 25 µL of *Bacillus licheniformis* protasis were added, and placed again in a thermostated bath at 60 ºC. After 30 min of heating, the solution was cooled to room temperature, to which 1 M hydrochloric acid was added dropwise to adjust the pH to 4.0
± 0.6. Finally, 10 µL of *Aspergillus niger* amyloglucosidase was added, and placed in a thermostated bath at 60 °C for 30 min. After cooling, four volumes of a 95:5 (*v/v*) ethanol:water solution were added and left at room temperature for approximately 18 h for complete precipitation of the insoluble fiber solution.

In previously calcined, weighed and identified filtration crucibles, suction was applied with a vacuum pump to completely remove residues (four crucibles were used). Then, the ethanol:water solution was filtered, quantitatively transferring the precipitate from the enzyme

solution to the crucible. Then, three successive washes of the residue were carried out with 20 mL of a 78:22 (*v/v*) ethanol:water solution, twice with 10 mL of a 95:5 (*v/v*) ethanol:water solution and twice with 10 mL of acetone (100%, complete drying of the residue). Then, the filtration crucibles were dried in an oven (Jouan, Berlin, Germany) at 105 °C to completely remove moisture. The dried samples were weighed, and of the two crucibles used, the residue from two of them was sent for ash determination and the other for crude protein determination (methods described above). The total dietary fiber content was determined following **Equation 4** and the values were expressed in g/100g dw.

Equation 4. Total dietary fiber content (g/100g dw).

$$Fiber\left(\frac{g}{100g}dw\right) = \left(\frac{(residue\ mass - protein\ mass - ash\ mass)}{sample\ mass}\right) * 100$$

4.6.6. Carbohydrates

The total carbohydrate content was estimated by difference and expressed in g/100g dw according to **Equation 5.**

Equation 5. Calculation of carbohydrate content (g/100g dw).

$$Carbohydrates\left(\frac{g}{100g}dw\right) = 100 - (Ash + fiber + fat + protein)$$

4.6.7. Energy Value

The energy value was calculated according to Regulation (EU) No 1169/2011 (2011) and expressed in kcal per 100 g of dry weight, as described in **Equation 6**.

Equation 6. Calculation of energy value (kcal/100 g dry weight).

$$Energy\left(\frac{kcal}{}dw\right) = (4 * (g\,protein + g\,carbohydrates)) + (2 * g\,fiber) + (9 * g\,fat)\ 100g$$

4.7. Chemical composition of commercial rocket plants

Alongside the nutritional assessment a chemical characterization was also carried out, namely assessment of the levels of tocopherols, organic acids, fatty acids and phenolic compounds, using various liquid chromatography and gaseous.

4.7.1. Organic acids

The determination of organic acids was performed by ultrafast liquid chromatography coupled to a diode detector (UFLC-DAD; Shimadzu Coperation, Kyoto, Japan) as previously described by Barros, Pereira, & Ferreira (2013a). For the extraction of organic acids, 1 g of sample was placed in a beaker, previously wrapped in aluminum foil, to which 25 mL of metaphosphoric acid (4.5%, v/v) were added. The mixture was placed under magnetic stirring for 20 min, at an ambient temperature of approximately 25 °C, and subsequently filtered into a 20 mL test tube. For chromatographic analysis, samples were filtered through 0.2 µm nylon filters into an amber vial (1.5 mL) for further analysis. Chromatographic analysis was performed on a Shimadzu 20A series UFLC system (Shimadzu Coperation, Kyoto, Japan). The separation of compounds was carried out using a C18 SphereClone reversed phase column (250 mm x 4.6 mm, 5 µm, Phenomenex), thermostated at 35 ºC. Detection occurred via a diode detector (DAD) using 215 nm and 245 nm (for ascorbic acid) as preferable wavelengths. The mobile phase used in isocratic mode was 3.6 mM sulfuric acid (H2SO4) using a flow rate of 0.8 mL/min. calibration curve: oxalic acid (y = 1×107x + 231891; R^2 = 0,9999; Limit of Detection (LD) = 6,3 µg/mL; Limit of Quantification (LQ) = 20,8 µg/mL); malic acid (y = 950041x + 6255,6; R^2 = 0.9999; LD = 15,9 µg/mL; LQ = 52,9 µg/mL);
ascorbic acid (y = 50000000 x 449262; R^2 = 0,9909 ; LD = 0,03 µg/mL; LQ = 0,11 µg/mL) and succinic acid (y = 50000000 x 449262; R^2 = 0,9995 ; LD = 0,29 µg/mL; LQ = 0,88 µg/mL). The results were express in mg/100 g dw.

4.7.2. Fatty acids

The fatty acid profile was determined by gas chromatography coupled to a flame ionization detector (GC-FID, DANI instrument model GC 1000, Milan, Italy), as described by (Barros, Pereira, Calhelha, et al., 2013b). The lipid extract resulting from Soxhlet extraction (see section 4.6.3.) was subjected to a transterification process to obtain FAMEs (Fatty acid methyl ester - fatty acid methyl esters). To this end, 5 mL of a methanol/sulfuric acid/toluene solution in a 2:1:1 ratio (*v/v/v*) was added to the total fat of the samples, and placed in a thermostated bath (Julabo, SW22; Seelbach, Germany) at 50 °C with stirring at 160 revolutions per minute (rpm) for 12 hours. Then, to recover the lipophilic phase, 3 mL of distilled water was added to the tubes, to obtain the different phases, and then 3 mL of ethyl ether with vortex stirring (LBX V05 series, LBX Instruments, LABBOX LABWARE S.L., Barcelona, Spain) for recovery of FAMEs. After phase separation, the supernatant was

transferred to a test tube with anhydrous sodium sulfate to dehydrate the supernatant, filtering, with a 0.2 µm nylon filter, into a 2 mL vial with a Teflon membrane cap. The individual fatty acid profile was obtained on a DANI 1000 GC system equipped with a split/splitless injector, flame ionisation detector (FID), with detector at 260 °C and a Macherey Nagel column (30 m × 0.32 mm × 0.25 µm df). The furnace temperature programme was as follows: the initial column temperature was 100 ºC for 2 min, then the temperature was increased at 10 ºC/min to 140 ºC, 3 ºC/min to 190 ºC, 30 ºC/min to 260 ºC for 2 min. The hydrogen (carrier gas) had a flow rate of 4.0 mL/min (0.61 bar), measured at 50 ºC. The split injection (1:50) was carried out at 250 ºC. 1 µL of the sample was injected for each analysis.

The identification of the fatty acids was based on the relative retention times of the peaks of the standard mixture of 37 FAMEs and the samples. The results were processed using Clarity 4.0.1.7 software (DataApex, Podohradska, Czech Republic) and expressed as a relative percentage of each fatty acid.

4.8. Statistical analysis

All chemical and nutritional assays were performed in triplicate, and the values were expressed as mean ± standard deviation (SD). Significant differences between samples were analyzed using the student's t-test with a 95% significance level, performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, New York, USA). In cases where more than two factors were analyzed, a one-way analysis of variance (ANOVA) was applied, followed by Tukey's HSD test with a = 0.05.

5. Results and discussion

5.1. Aquaculture system

The decision to use the Aquaneering System for the aquaponic project, featuring *Squalius alburnoides* (calandino) fish and rocket plants, is grounded in both sustainability and system efficiency. This particular system, which operates as a recirculating aquaculture system (RAS), stands out for its ability to conserve water and create a closed-loop environment where waste is minimized, and nutrients are recycled. The Aquaneering System allows precise control over key parameters such as water quality, oxygenation, and temperature, which ensures both the fish and plants thrive (Bernardo et al., 2008). Squalius alburnoides was the most appropriate choice for this system due to its resilience and adaptability to fluctuating water conditions, which can often challenge other species. As a native species to the Iberian Peninsula, it is well-suited to the environmental conditions in Portugal, and using it promotes biodiversity conservation, a key consideration in sustainable aquaponics.

Technologically, the Aquaneering System's design is optimized for ease of management, with integrated monitoring tools and filtration systems that allow for fine-tuning conditions, ensuring a stable environment for both the fish and plants. This provides a practical advantage over conventional aquaculture systems, which often struggle with waste management and environmental impact. By combining aquaculture and hydroponics, this system creates a sustainable and highly productive setup.

The experimental evaluation of *Squalius alburnoides* (calandino) in the Aquaneering system yielded limited growth, with notable differences between initial and final parameters, as shown in **Figure 14**, that underscore the impacts of environmental and water quality challenges. At the experiment's start, fish lengths averaged between 5.7 and 6.0 cm, with weights between 1.6 to 2.3 grams, reflecting the baseline conditions. Corresponding q-factors (condition factors) varied from 0.82 to 1.18, indicating a range of initial health and growth potential across the cohort (**Figure 15**).

By the conclusion of the trial, some fish demonstrated mild growth, with lengths extending to approximately 6.4 cm and weights reaching up to 3.8 grams for select individuals. The final q-factors also showed some increases, particularly for fish with improved weights, with final q-factors ranging from 0.92 to 1.45 (**Figure 16**). This variation

in q-factor indicates that while some fish responded well to the aquaculture setup, others showed minimal growth, suggesting inconsistencies likely tied to environmental stressors.

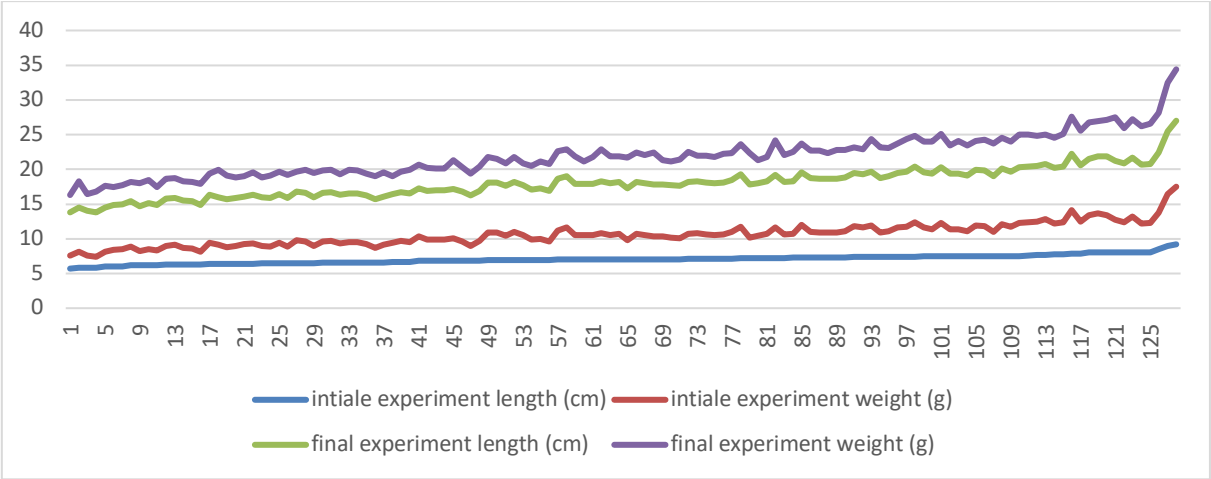


Figure 14. Length (cm) and weight (g) progression of caladino fish during experimental period.

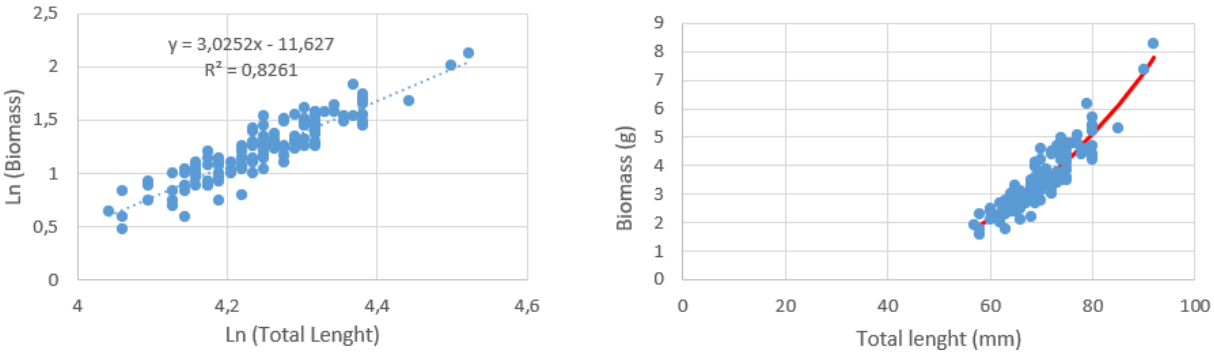


Figure 15. Relationship between biomass and total length of calandino fish in the initial experiment.

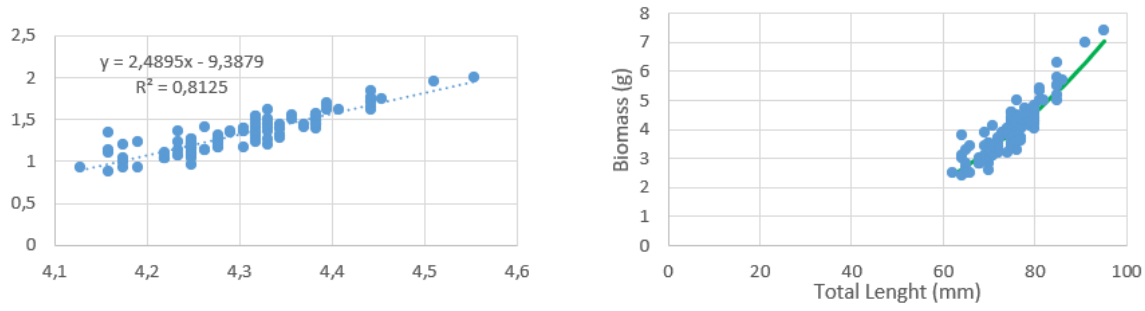


Figure 16 Relationship between biomass and total length of calandino fish in the final experiment.

One of the most critical factors impacting growth is the water's conductivity. Measurements indicated values consistently over 500 µS, markedly higher than the 50 µS typical of *S. alburnoides*' natural habitats. This elevated conductivity level could lead to

osmotic stress, which in turn affects growth rates, as the fish must expend more energy on osmoregulation rather than growth. The high conductivity may also negatively impact gill function, impairing the fish's ability to efficiently absorb oxygen, nutrients, and maintain ionic balance.

Comparing these results with findings from similar studies, a study by Santos et al. (2020) on the growth performance of *Squalius alburnoides* in aquaculture systems, highlights the importance of maintaining environmental conditions that closely mimic the species' natural habitat. In their study, fish cultured in tanks with conductivity levels below 100 µS and minimal pathogen presence achieved higher growth rates and more consistent q-factors, averaging around 1.2 across the cohort, which indicates healthier overall conditions. In contrast, our experiment faced elevated conductivity (over 500 µS) and pathogen contamination (salmonella and coliforms), which likely inhibited growth and led to inconsistent q-factors across individuals. This comparison underscores the significance of water quality, particularly conductivity and pathogen management, as key determinants of growth success in controlled aquaculture systems for *S. alburnoides*.

5.2. Hydroponic system

Rocket seeds were germinated in phenolic foam for 5 days in the dark at room temperature (20 – 25 ºC). When necessary, the seeds were watered with tap water (pH around 5.5 – 6.7 and conductivity between 600 – 700 µS. In the IPB greenhouse, a hydroponic channel was installed with four plugged holes in a closed system (as shown in section 4.5.2, **Figure 12**) where, after germination, 48 young rocket plants were transplanted, of which 32 were watered with wastewater from the aquaculture and 16 were watered with tap water.

The first challenge was the change in conditions. In the greenhouse, there were noticeable shifts in temperature and humidity compared to the previous phase. Rocket plants, which generally prefer cooler (24-26°C), stable environments, had trouble adjusting to these changes. Since the system was closed, we manually watered the plants weekly with nutrient-rich fish water, recording data on pH, conductivity, temperature as shown in the **Table 5** and the plant height, and leaf count (**Figure 17**). Despite our efforts, the real problem started as temperatures in the greenhouse began to rise beyond the plants' tolerance.

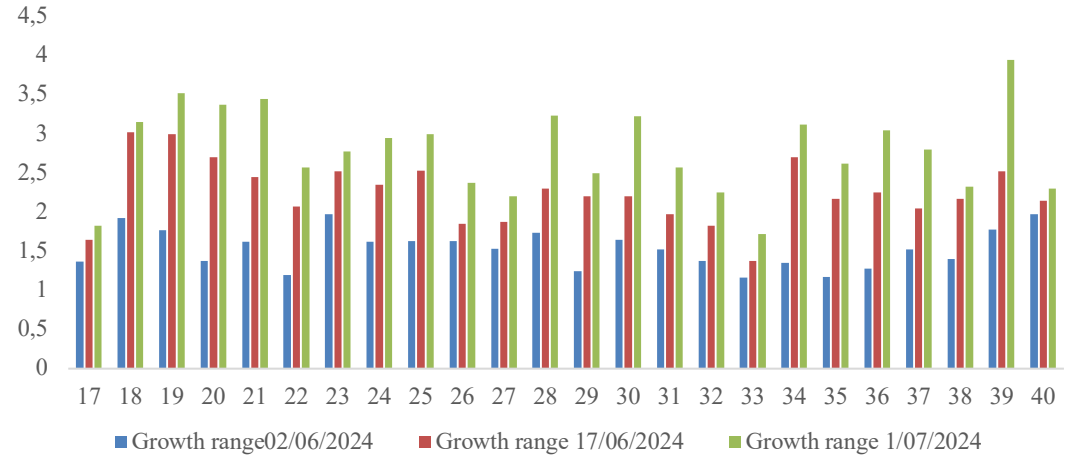
Table 5. Weekly environmental parameters summary: temperature, pH, and conductivity for fish and control lines.

Weeks	Lines	Temperature	PH	Conductivity
Week 0	Fish	17,2 [18-16,3]	8,55 [8,8-8,3]	580 [600-560]
	Control	16,2 [16,3-16,1]	8,7 [8,9-8,4]	571 [594-548]
Week 1	Fish	21 [21,4-20,6]	8,2 [8,3-8,1]	589 [615-526]
	Control	20,9 [21,6-20,2]	8,3 [8,5-8,1]	571 [616-526]
Week 2	Fish	21,3 [22,3-20,2]	8,2 [8,5-7,9]	585 [643-526]
	Control	21,3 [21,4-21,2]	8,2 [8,2-8,1]	564 [641-487]
Week 3	Fish	21,5 [21,8-21,2]	8,1[8,2-8]	473 [647-298]
	Control	21,4 [21,5-21,2]	8,4 [8,5-8,2]	463 [605-321]
Week 4	Fish	22,7 [23,9-21,4]	7,8 [8,1-7,4]	596 [671-520]
	Control	21,9 [22,2-21,5]	8,1 [8,2-7,9]	620 [670-570]
Week 5	Fish	22 [22,5-21,4]	8 [8,1-7,9]	626 [678-674]
	Control	23,6 [25,6-21,6]	8,1 [8,2-8]	605 [677-532]
Week 6	Fish	22,4 [23,2-21,5]	8,2 [8,3-8]	457 [613-300]
	Control	23,4 [24,9-21,9]	8,4 [8,5-8,2]	587 [640-534]
Week 7	Fish	21,8 [22,5-21]	8,1 [8,4-7,8]	618 [636-600]
	Control	22,4 [23,4-21,3]	8,3 [8,4-8,1]	604 [634-574]
Week 8	Fish	22,3 [23,4-21,2]	8,2 [8,4-8]	458 [661-255]
	Control	23,7 [26,3-21]	8,4 [8,6-8,1]	638 [660-615]
Week 9	Fish	21,6 [22,6-20,5]	7,9 [8,1-7,7]	688 [691-684]
	Control	20,7 [21-20,3]	8,2 [8,2-8,1]	679 [686-672]
Week 10	Fish	20,6 [21-20,2]	8,3 [8,8-7,8]	688 [682-293]
	Control	20,5 [20,8-20,2]	8,6 [8,8-8,3]	678 [683-673]

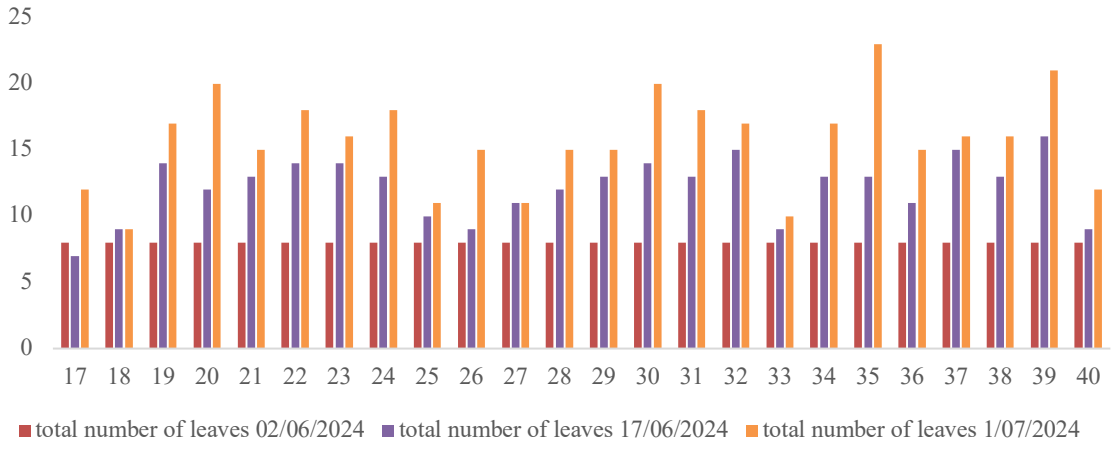
Unfortunately, the temperature in the greenhouse exceeded the limits rocket plants can handle (24-26°C), and this increase in heat led to a spike in humidity as well (50-70%). The combination of high heat and high humidity became too much for the plants to survive. Even though the efforts to manage the conditions and save the plants, the environment was simply too harsh for them. Over the course of 5 to 9 weeks, it became clear that the excessive heat and humidity were causing stress to the plants, leading to poor growth and eventually plant death.

This experience highlighted a major issue with the hydroponic setup: the lack of adequate climate control in the greenhouse. It was underestimated how hot it would get, and without proper ventilation or cooling systems, the plants couldn't cope. Despite monitoring and adjusting variables like water and nutrients, the extreme temperatures were the primary cause of failure in this experiment. This shows how crucial it is to keep environmental conditions in check, especially when dealing with plants like rocket that are sensitive to heat.

Additionally, considering the plant's sensitivity to heat, conducting experiments during cooler seasons or modifying the protocol to include more temperature-resilient plants might yield better results.



A



B

Figure 17. Average length of each plant group (17-40) (**A**); total number of leaves per plant group (17-40) (**B**).

5.3. Deep water cultivation system

An alternative for greater control of cultivation parameters was the preparation of hydroponic systems in deep water on the premises of the Centro de Investigação de Montanha (CIMO). Germination took place as described above; after 5 days, the rocket plants were transplanted into the systems. In this experiment, two deep water hydroponic systems were used to grow rocket plants, each presenting distinct challenges that ultimately affected the viability of the seedlings. The first system relied on fish water to supply natural nutrients; before introducing the seedlings, the initial pH and electrical conductivity (EC) were measured to ensure compatibility with rocket plants' requirements. Rocket plants grow best in water with a stable pH range of 5.5–6.5 and an EC of 1.6–2.4 mS/cm. However, due to the biological variability in fish water, there were frequent fluctuations in both pH and conductivity, making it difficult to maintain these ideal ranges. The young seedlings, only five days old, had insufficiently developed roots to efficiently absorb nutrients, making them highly sensitive to these fluctuations. Additionally, environmental conditions in the growth area saw temperature variations beyond the ideal 15–20°C range for rocket plants. Elevated temperatures likely increased the seedlings' respiration rates, placing further stress on their delicate tissues. As a result, these factors compounded to create an environment where the seedlings could not thrive, leading to poor growth and eventual plant failure (Moreno, Suarez, et Garcia 2021).

In the second system, it was attempted to provide a more controlled setup using distilled water with a nutrient solution. By removing the variability of fish water, it was aimed to control nutrient levels more precisely, using a growth-focused nutrient solution. Based on reference recommendations, it was prepared a 1:600 dilution rate, adding 1 ml of solution per 600 ml of distilled water to achieve the target nutrient concentration. However, the young seedlings, without established roots, proved unable to handle even this concentration, showing signs of nutrient burn shortly after introduction. Distilled water, while free from contaminants, lacks the buffering capacity of natural or fish water, which may have made the solution's nutrient salts more aggressive on the delicate rootless plants. Furthermore, high temperatures in the room likely accelerated evaporation, increasing nutrient concentration over time and exacerbating stress on the seedlings. In retrospect, these young rocket plants would have benefitted from a gentler, lower-concentration nutrient solution, along with an acclimatization period in a nursery or gentler hydroponic system to develop root structures (Hamza et al. 2022).

In future iterations, several refinements could improve outcomes. Allowing seedlings to mature to around 2–3 weeks before transitioning to hydroponic systems would support stronger root development, improving resilience. Maintaining consistent environmental conditions, especially with stable temperatures in the range of 18–22°C and humidity at 50 – 60% would also help. For pH-sensitive plants like rocket, regular monitoring and buffering could stabilize water quality, enhancing nutrient availability and uptake. A nursery phase, with either rockwool or a less intense nutrient solution, could allow gradual adaptation, making plants better suited to thrive in both fish-based and distilled water hydroponic systems (Saaid et al. 2013).

5.4. Quality of the water

The concentration ranges (mg/L) of phosphates, nitrites, nitrates and dissolved oxygen in water suitable for irrigation according to ISO 10304-1:200, 13395:1996, 15705:2002, and 6878:2004, are described in **Table 6**. The results found in the study for the same parameters are presented below.

Table 6. ISO guidelines on water quality (Barbosa et al. 2015).

Parameter	ISO Recommended Level (General Range)
Phosphates	< 5 mg/L
Nitrites	< 0.5 mg/L
Nitrates	< 50 mg/L
Dissolved Oxygen	≥ 3-5 mg/L

5.4.1. Phosphates

In evaluating the average phosphate levels in the water from the aquaculture system, it was possible observe some interesting trends in the fish and control lines. The fish lines began with an average phosphate level of 0.0238 mg/L at T0, which rose to 0.0566 mg/L at T1 but then dropped to 0.0131 mg/L at T2. There was a notable increase to 0.0364 mg/L at T3, culminating in a significant spike to 0.3180 mg/L at T4. On the other hand, the control samples started at 0.0241 mg/L at T0, increased to 0.0477 mg/L at T1, dipped slightly to 0.0123 mg/L at T2, and then showed a small rise to 0.0353 mg/L at T3, finishing at 0.3015 mg/L at T4. While this lower level may seem advantageous at first glance, it highlights a troubling aspect of the aquaculture system: the plants are not thriving as anticipated. The

reduced phosphate levels suggest that there may not be enough available nutrients for the plants, which is likely contributing to their poor growth and overall lack of results. In conventional systems, higher phosphate levels can lead to more robust plant growth, but in this case, the filtration processes of the aquaculture setup seem to be inhibiting nutrient availability rather than enhancing it. This scenario underscores the need for careful monitoring and adjustments in nutrient management. The phosphate levels across the different water sources and their implications for plant growth are illustrated in the **Figure 18** where the two illustrations of the water fish and the control are nearly the same with a maximum concentration of 0.3 mg/L and below the appropriate concentration that we need for the plants to grow properly.

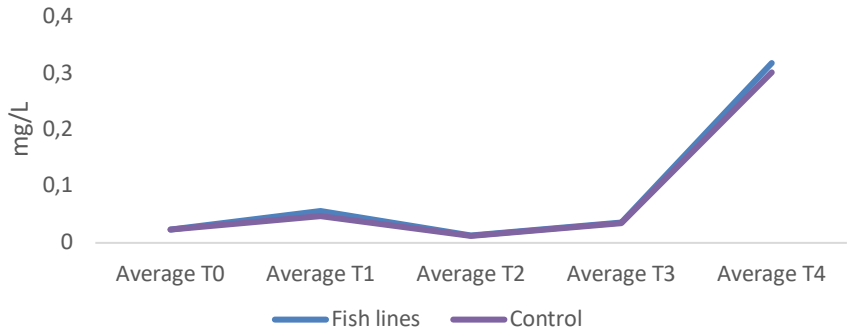


Figure 18. Phosphate concentration (mg/L) over time during experiment.

5.4.2. Nitrites

In examining the average nitrite levels across different samples in the aquaculture system, both the fish and control lines demonstrated consistently low concentrations, which is concerning for plant growth. The fish water started with an average nitrite level of 0.058 mg/L at T0, which declined to 0.033 mg/L at T1, in T2 and T3 dropped to unquantifiable levels of the calibration line used, before experiencing a slight recovery to 0.0289 mg/L at T4. Similarly, the control water began at 0.0579 mg/L at T0, decreased to 0.0234 mg/L at T1, reached unquantifiable levels as in the fish water in T2 and T3, and ended at 0.0211 mg/L at T4.

These consistently low nitrite readings indicate that the nitrogen cycle in the aquaculture system may not be functioning as it should, which likely contributes to nutrient deficiencies for the plants.Although lower nitrite levels can sometimes suggest a balanced aquaponic environment, in this case, they seem to be stunting the plants growth and overall health. This situation underscores the need for better management of nutrient levels to ensure

that the system can support healthy plant development. The nitrite levels measured over the different time points are summarized in **Figure 19.**

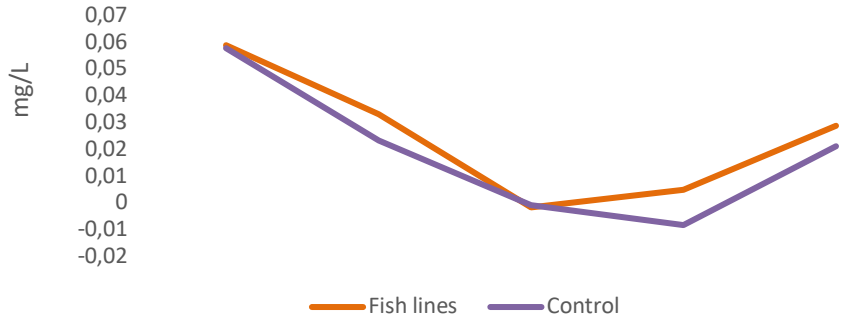


Figure 19 Nitrites concentration (mg/L) over time during experiment.

5.4.3. Nitrates

In analyzing the average nitrate levels from the aquaculture system, both the fish wastewater and control water exhibited variable results across the different time points. The fish wastewater recorded an average nitrate level of 41.46 mg/L at T0, which increased significantly to 67.87 mg/L at T1 before dropping to 26.06 mg/L at T2. The levels then rose again to 58.46 mg/L at T3 and settled at 50.37 mg/L by T4. In comparison, the control water started at 43.83 mg/L at T0, peaked at 67.68 mg/L at T1, then decreased to 28.25 mg/L at T2, followed by a reduction to 55.85 mg/L at T3 and finishing at 51.61 mg/L at T4.

When comparing these results with the ISO standards (**Table 6**) for acceptable nitrate levels in aquaculture systems, which typically recommend keeping nitrate concentrations below 50 mg/L to avoid potential toxicity to aquatic life, it's concerning to see that the fish lines exceeded this threshold during certain periods. Although the levels in both the fish and control lines fluctuate, the overall nitrate concentrations raise questions about the health of the system and its ability to sustain optimal plant growth. These fluctuations indicate a need for careful monitoring and management of nutrient levels to align with ISO recommendations and ensure the health of both the aquatic life and the plants in the system. The nitrate levels across the various time points are summarized in **Figure 20**.

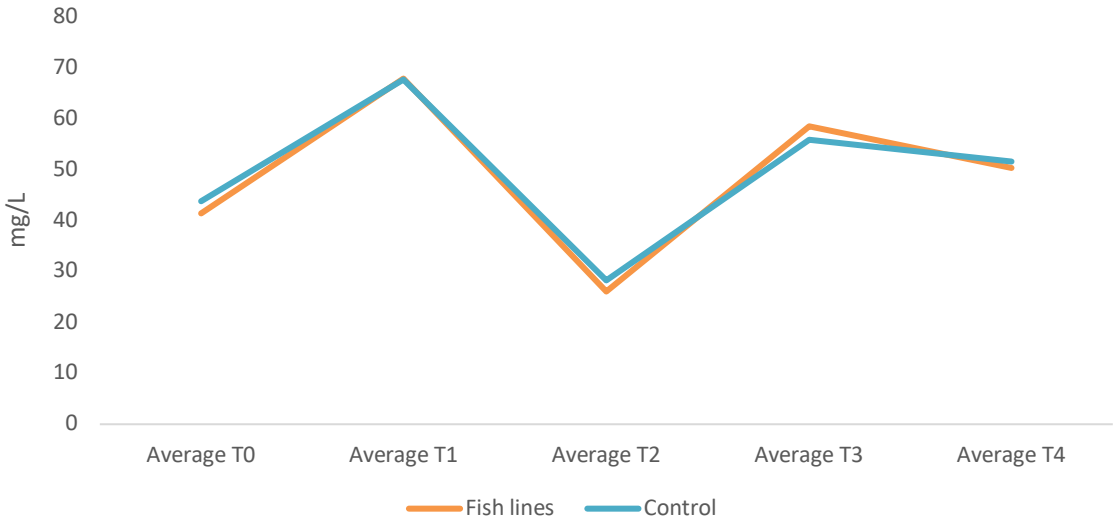


Figure 20. Nitrates concentration (mg/L) over time during experiment.

5.4.4. Oxygen levels

In assessing the average dissolved oxygen levels in the aquaculture system, it was possible to check a notable difference between the fish wastewater and control water. The fish wastewater started with an average oxygen level of 0.428 mg/L at T0, which slightly increased to 0.436 mg/L at T1 and 0.456 mg/L at T2. However, there was a significant jump to 0.692 mg/L at T3, followed by a slight decrease to 0.648 mg/L at T4. In contrast, the control water showed consistently higher levels, starting at 0.584 mg/L at T0 and increasing to 0.568 mg/L at T1. They peaked at 0.688 mg/L at T2, followed by an impressive rise to 0.760 mg/L at T3 and reaching 0.952 mg/L at T4.

Comparing these results to ISO standards (**Table 6**) which recommend maintaining dissolved oxygen levels between 3 and 5 mg/L for optimal health of aquatic organisms, it's evident that the fish wastewater and control water are significantly below this threshold. This raises concerns about the well-being of the fish in the system, as low oxygen levels can lead to stress and hinder growth. The control samples, while higher than the fish lines, still fall short of the ideal levels. These findings highlight the need for improved aeration and monitoring strategies within the aquaculture system to ensure that both the fish and plants can thrive. The oxygen levels measured across the various time points are summarized in **Figure 21**.

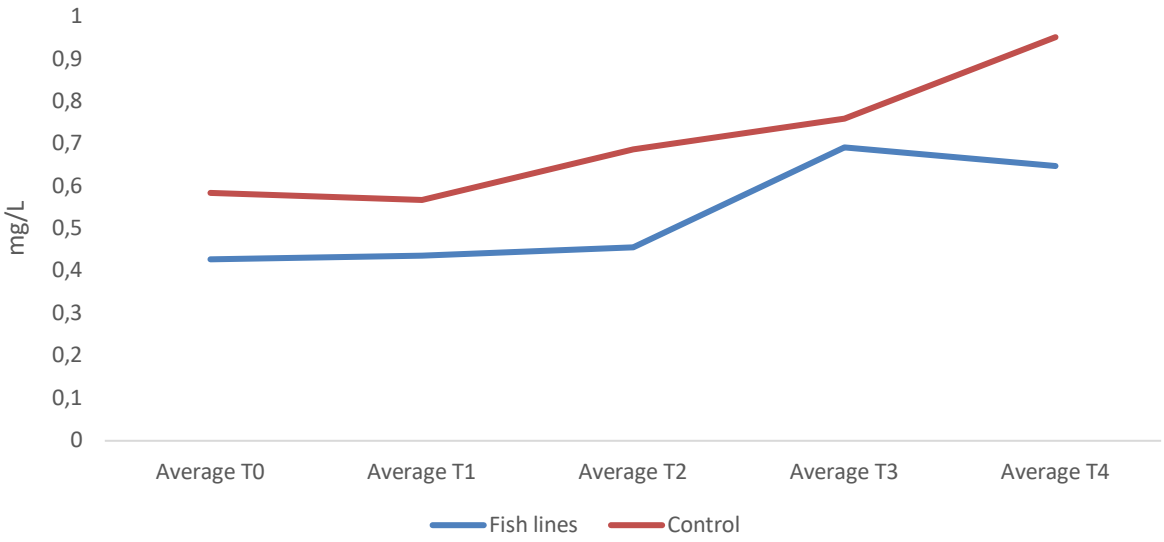


Figure 21. Oxygen concentration (mg/L) over time during experiment.

5.5. Microbiology

5.5.1. Count of cultivable microorganisms

The microbial testing of fish wastewater samples revealed significant differences in bacterial growth based on temperature and dilution. At both 22 °C and 37 °C, undiluted samples displayed unmanageable bacterial overgrowth, indicating a high initial microbial load. Therefore, serial dilutions were conducted to facilitate the enumeration of colonies as shown in **Table 7** and **Figure 22**. At 22°C, the bacterial colonies in the diluted samples showed a notable decrease in count: the 0.01 dilution yielded 44 – 45 Colony Forming Units (CFU), while the 0.001 dilution yielded only 5 – 8 CFU. This result aligns with the fact that 22 °C is slightly lower than the optimal growth range for many bacteria, which slowed their proliferation. In contrast, the bacterial counts at 37 °C, a more favorable temperature for mesophilic bacteria, were considerably higher. At this temperature, the 0.01 dilution had a CFU range of 25 – 67, while the 0.001 dilution ranged from 15 – 28 CFU. These differences suggest that higher temperatures accelerate bacterial activity, likely due to enzyme efficiency and increased metabolic rates in mesophilic species commonly found in aquatic environments.

The control water further highlighted the distinct bacterial profile of fish wastewater. At 22 °C, there was no bacterial growth in any of the control water, confirming that environmental contamination was minimal. At 37 °C, the undiluted control sample showed a slight growth (97 – 99 CFU), but this was likely from minimal background contamination, not representative of the rich microbial load in the fish wastewater.

Table 7. Microorganism count across multiple dilutions.

	Temperature					
	22°C			37°C		
Dilution	1	0.01	0.001	1	0.01	0.001
Fish wastewater	Uncountable	44 – 45	5 – 8	Uncountable	25 – 67	15 – 28
Control water	Nothing	0	0	97 – 99	0	0

These findings are consistent with literature on microbial communities in aquaculture, where nutrient-rich fish environments foster diverse and abundant bacterial populations. This high bacterial load is essential for nutrient cycling but can pose a risk to system balance if not managed. In aquaponic systems, for example, understanding these bacterial dynamics helps optimize conditions for fish health and plant growth by informing proper water treatment and microbial management practices.

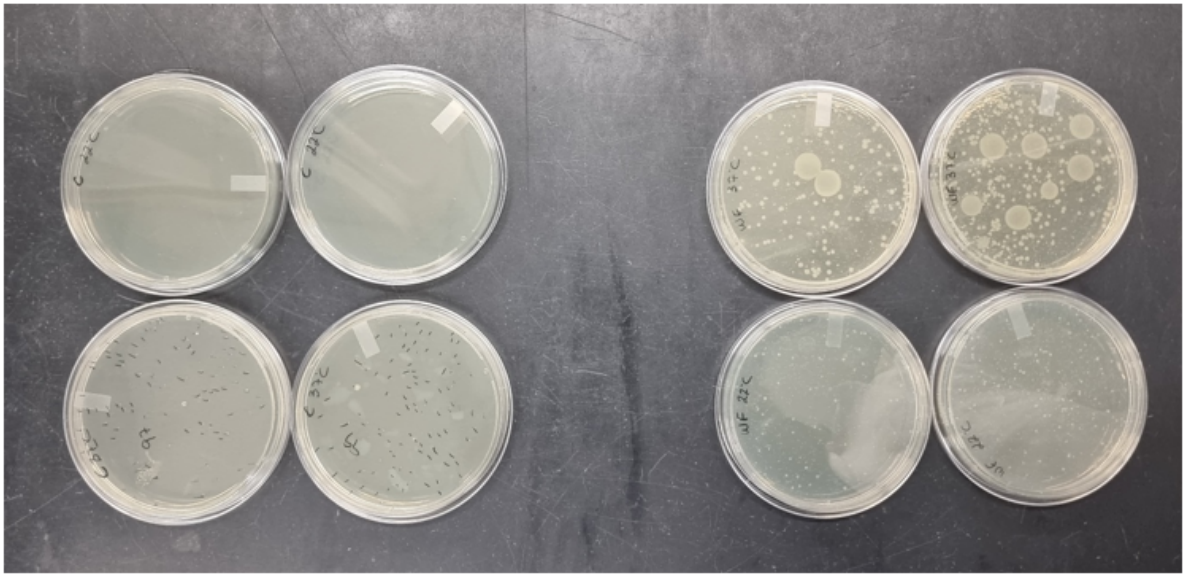


Figure 22. Culture plates showing microorganism colony counts.

5.5.2. Analysis of Clostridium perfringens

Regarding the analysis of *Clostridium perfringens* of fish waste water and control water, it was used selective media designed to detect the bacteria, expecting any colonies present to show a black coloration due to the sulfite reduction reaction characteristic of this bacteria. However, no black colonies were observed on any of the plates **(Figure 23),** for either the fish wastewater or the control water. This absence suggests that *C. perfringens* was not present at detectable levels in both waters, indicating that the fish water environment did

not support its growth, or that its population was below the sensitivity threshold of the test. These findings align with expectations in well-maintained aquaponic systems, where anaerobic pathogens are generally low due to aeration and regular water circulation.

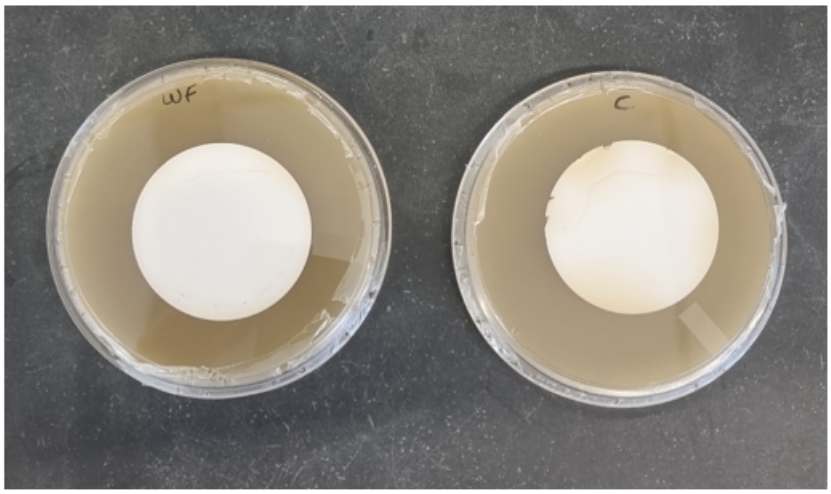


Figure 23 Culture plates showing *Clostridium perfringens*. WF – fish wastewater and C – control water.

5.5.3. Total coliforms analysis

During the microbiological assessment of the fish wastewater, we found that total coliforms were present in colonies so large that it was impossible to count them accurately. The sheer volume of these colonies exceeded the counting capacity, indicating a potentially serious contamination problem at the water source. Recognising the importance of quantifying these bacteria for health in the aquaponic system, serial dilutions at concentrations of 0.01 and 0.001 were proposed as describe in **Figure 24**. This approach provided a clearer picture of the microbial load in the water.

Through this dilution process, up to five different types of coliforms were identified, each of which poses varying risks to plant growth and the general ecosystem of aquaponic systems. The colour of the colonies varied, with some exhibiting a yellow hue that is normally characteristic of certain coliform species, while others appeared greenish, which can indicate faecal contamination. This variation in the colour of the colonies further highlights the complexity of the contamination in the aquaculture system. The presence of these coliforms is particularly worrying as they are often associated with pathogens that can negatively affect plant health and water quality. The findings highlighted the delicate balance needed to maintain a healthy aquaponic system, in which the health of fish and plants depends on the microbiological integrity of the environment they share.

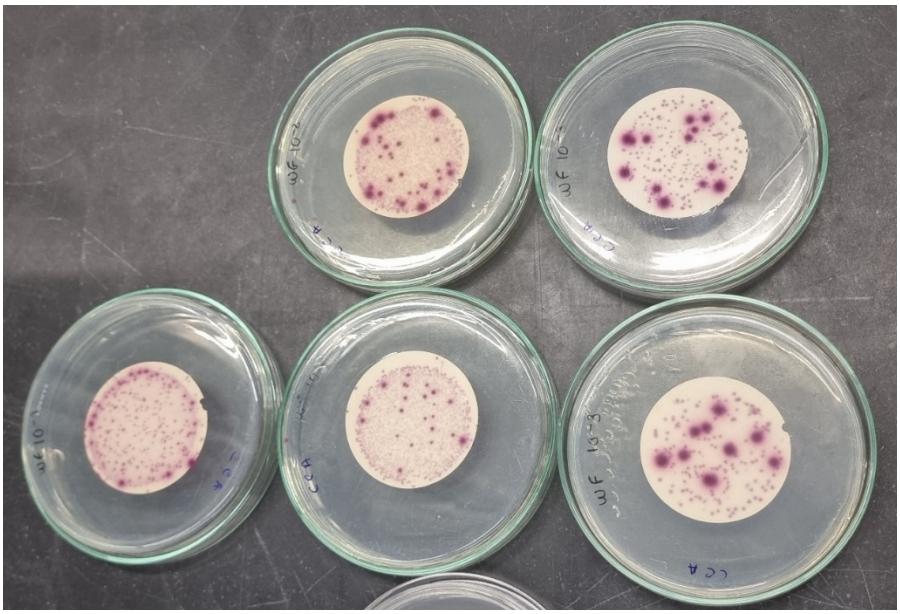


Figure 24. Culture plates with coliform colonies.

5.6. Nutritional profile

The commercial rocket leaves were analysed for their nutritional profile (total fat, ash, crude protein, fibre, carbohydrates) and chemical composition in terms of organic and fatty acids as shown in **Table 8,** since the primary aim was to compare these values with rocket from aquaponic systems. However, even though the aquaponic plants were not obtained, the profile of the commercial leaves was presented to compare them with literature that cultivated rocket or *Brassicas* in aquaponic systems. The findings of commercial rocket leaves are described below.

The total fat content in the commercial rocket plant samples was analyzed and found to be approximately 4.8 ± 0.2 g/100g on a dry weight (dw) basis (average ± standard deviation). This level of fat content is typical for leafy greens, as rocket plants generally possess a modest lipid profile, providing essential fatty acids without significantly contributing to dietary fat intake.

Studies on rocket plants cultivated in aquaponic systems often report comparable or slightly higher fat content due to the nutrient-rich environment that may promote lipid accumulation. Research indicates that aquaponic rocket plants can contain up to 5.0 – 5.2 g/100g dw of fat, slightly exceeding the levels observed in soil-grown counterparts (Kafkas et al. 2017). This difference could stem from the continuous nutrient availability in aquaponic systems, particularly if enriched with organic fish by-products that may enhance lipid synthesis.

Table 8. Nutritional profile (total fat, ash, crude protein, fiber, carbohydrates and energy) and chemical composition in terms of organic acids and fatty acids of commercial rocket plants (Media±SD).

Nutritional profile (fresh weight)	
Total fat (g/100 g)	0.45±0.02
Crude protein (g/100 g)	3.4±0.1
Ash (g/100 g)	1.74±0.02
Total fiber dietary (g/100 g)	3.33±0.03
Carbohydrates (g/100 g)	0.5±0.1
Moisture (%)	90.59±0.01
Energy (kcal/100 g)	26.3±0.1
Organic acids (mg / 100 g of fresh weight)	
Oxalic	3.9±0.3
Malic	0.24±0.002
Ascorbic	0.01±0.0002
Succinic	1.6±0.1
Total	**5.7±0.3**
Fatty acids (%)	
C6:0	95.05±0.03
C17:0	0.753±0.001
C17:1	0.09±0.01
C18:3n3	0.75±0.01
C20:1	2.76±0.01
C20:5n3	0.07±0.001
C24:1	0.525±0.004
SFA	**95.81±0.03**
MUFA	**3.37±0.03**
PUFA	**0.82±0.01**

The ash content in commercial rocket plant samples was measured at 18.5 g/100g on a dry weight basis. This level reflects the total mineral content in the plant tissue, a crucial indicator of the presence of essential inorganic nutrients such as potassium, calcium, and magnesium, which play vital roles in plant growth and human nutrition.

In aquaponic systems, where nutrient recycling is optimized, rocket plants may show higher ash content due to the continuous availability of dissolved minerals from fish waste. Literature reports ash contents for aquaponic rocket ranging from 19.0 to 20.0 g/100g dw, which is slightly higher than that in soil-grown varieties (Goddek et al., 2019). This elevated mineral concentration is often attributed to the aquaponic nutrient cycle, enriching plants with readily absorbable minerals throughout their growth cycle.

The commercial rocket plant also demonstrated a high crude protein content, with an average of 36.2% on a dry weight basis. This substantial protein percentage positions rocket as a favorable option for protein intake, especially among leafy greens. Protein levels in plants like rocket can be influenced by several factors, including soil quality, nutrient availability,

and environmental conditions. The impressive protein content suggests that this commercially grown rocket could be a valuable dietary component, particularly in vegetarian and vegan diets where plant-based proteins are prioritized.

When comparing this commercially grown rocket's protein content with that of rocket plants cultivated in aquaponic systems, studies indicate that aquaponics can result in comparable or sometimes enhanced protein levels due to nutrient-rich water from fish waste. For instance, Rakocy et al. (2006) observed that leafy greens in aquaponics often exhibit elevated nutrient profiles, including higher protein, due to the continuous nutrient recycling typical of these systems. However, variability in protein content might still arise due to differences in fish species, feed, and system design. In the current study, if the aquaponic system yields slightly lower protein percentages, factors such as plant age and water quality could contribute to these differences.

The fiber content in the commercial rocket plant was measured at approximately 35.6% on a dry weight basis. This high fiber content underscores the plant's suitability for diets emphasizing digestive health, as dietary fiber aids in promoting satiety, stabilizing blood sugar levels, and maintaining gut health. Rocket plants, known for their relatively tough leaf texture, naturally contain high fiber levels, which could benefit those looking to increase their dietary fiber intake. Fiber content in aquaponically grown rocket plants is generally consistent with that of commercially grown plants, though slight variations can occur based on growth conditions and nutrient availability in the system.

Research by Goddek et al. (2015) suggests that aquaponic conditions may support robust fiber formation due to optimal moisture and nutrient levels. Thus, while commercial systems produce high-fiber rocket, aquaponic systems are likely capable of yielding similar fiber concentrations, which may be influenced by parameters like pH and nutrient ratios. The commercial plants contain 35.4 ± 0.3 g/100g dw of fiber, which reflects the carbohydrate content as part of the plant's total dry matter. Fiber levels in commercially grown rocket are typically influenced by consistent nutrient delivery, supporting regular carbohydrate accumulation.

Aquaponic systems provide nutrients that can enhance the overall carbohydrate profile, potentially increasing fiber and other carbohydrate content. Studies have shown that plants in aquaponic systems can have slightly elevated carbohydrate levels due to optimal nutrient uptake and the symbiotic relationship with microbial populations. This can increase the fiber content by approximately 10-15% compared to conventionally grown counterparts. The aquaponic environment's optimized nutrient profile contributes to higher carbohydrate levels,

as indicated by increased fiber content. Enhanced fiber content in aquaponic rocket plants offers health benefits, appealing to consumers looking for fiber-rich diets. This could position aquaponic produce as a premium choice for health-conscious markets (Krastanova et al. 2022).

In commercial settings, maintaining optimal humidity is vital for reducing stress and improving plant yield. Commercial rocket plants are generally kept in humidity-controlled environments to manage water loss and prevent disease. Although we don't have the exact humidity data from this dataset, typical commercial setups aim to keep relative humidity between 50-70%. In aquaponic systems, humidity levels may fluctuate slightly higher due to the water-intensive nature of the environment. Studies indicate that the levels can often reach up to 75%, as the water from aquaculture and transpiration from both plants and fish contribute to increased moisture in the air. This humidity level benefits the growth environment but may demand more precise humidity control strategies. Aquaponic systems offer an environment with naturally high relative humidity, which might support certain plant species better by reducing water stress. However, this variability can pose challenges for maintaining consistent humidity, potentially leading to higher disease risk without effective control measures compared to commercial rocket plants.(Siomos et Koukounaras, 2007) The commercial rocket plants show an energy content of 279.1 kcal/100g dry weight (dw), which reflects a typical nutrient density suitable for market demands. In controlled environments, commercial growers can optimize light, temperature, and nutrition to achieve consistent energy levels, supporting consumer expectations for taste and caloric content.

Aquaponic systems often enhance the nutrient profile of plants, which can result in increased energy content. Literature on aquaponic-grown leafy greens, including rocket, suggests that plants in aquaponic systems can have slightly higher energy values due to the balanced nutrient availability from fish waste and microbial activity, which promote robust growth and nutrient accumulation. The energy content of aquaponic rocket plants may surpass commercial plants slightly due to the nutrient-dense aquaponic solution, providing more complex nutrients compared to standard commercial fertilizers. This results in minor yet meaningful nutritional gains, presenting aquaponics as an option for more nutrient-enriched produce (Miyazawa, Maehara, et Kurose 2002).

6. Conclusions

This study highlights the challenges and critical factors that impacted the performance of the aquaponic system featuring *Squalius alburnoides* and rocket plants, underscoring the need for more rigorous control of environmental and water quality parameters. Despite the Aquaneering System's advanced design and sustainability-oriented approach, several key issues affected a suitable environment for both fish and plants.

For the aquaculture component, low levels of nitrites, phosphorus, and dissolved oxygen proved detrimental to fish health and growth. Nitrite levels were consistently below optimal thresholds, indicating a disrupted nitrogen cycle that limited nutrient availability. Phosphorus levels, critical for both fish metabolism and plant growth, fluctuated significantly and remained insufficient to support robust growth. Dissolved oxygen levels were alarmingly below ISO-recommended ranges, creating a stressful environment for the fish and impairing their ability to thrive. Additionally, the high microbial load, including the presence of salmonella and coliforms, impose more challenges, representing a risk to fish health and nutrient stability in the system. Elevated water conductivity, over five times the levels found in the species' natural habitat, further exacerbated stress, conducting energy from growth to osmoregulation.

For the hydroponic component, inconsistent control of key environmental variables severely affected rocket plant growth. The greenhouse setup lacked adequate temperature and humidity regulation, exposing plants to conditions well outside their optimal range. Excessive temperatures and humidity levels caused significant stress, ultimately leading to plant failure. Similarly, fluctuations in pH and electrical conductivity (EC) in the aquaponic water created an unstable nutrient environment for the plants. Young seedlings, with underdeveloped roots, were particularly sensitive to these variations, further compromising their ability to absorb nutrients effectively. Even in the controlled deep-water hydroponic systems, challenges such as nutrient burn, rapid evaporation, and the absence of buffering capacity in distilled water revealed the importance of fine-tuning nutrient concentrations and acclimatization protocols.

These findings emphasize the critical need for rigorous control of environmental parameters in aquaponics. For future implementations, maintaining stable and optimal conditions for pH, EC, temperature, and oxygenation is essential. Introducing additional measures such as effective aeration, microbial management, and improved climate control systems would enhance the stability of the aquaponic environment. In the hydroponic phase, gradual acclimatization of seedlings and using gentler nutrient solutions could significantly

improve plant resilience and growth outcomes. Finally, optimizing the system for the specific requirements of both the fish and plants, including aligning conductivity and nutrient levels with their natural tolerances, is crucial for achieving a sustainable and productive aquaponic system.

The comprehensive analysis of fish wastewater, microbial activity, and the nutritional profile of commercial rocket leaves highlights critical factors for optimizing aquaponic systems. Fish wastewater exhibited significant microbial activity, with bacterial growth varying based on temperature and dilution. At 22 °C, bacterial counts were lower (44-45 CFU at 0.01 dilution; 5-8 CFU at 0.001 dilution), reflecting suboptimal growth conditions for many bacteria. In contrast, 37 °C promoted higher microbial activity (25-67 CFU at 0.01 dilution; 15-28 CFU at 0.001 dilution), indicative of mesophilic bacteria thriving under favorable conditions. Control water samples demonstrated minimal microbial presence, underscoring the nutrient-rich nature of fish wastewater as a microbial hotspot. These findings underscore the need for proper microbial management to maintain water quality and system balance in aquaponics. No evidence of Clostridium perfringens was detected in either the fish wastewater or control water, suggesting that the aquaponic environment inhibited the growth of anaerobic pathogens. However, the fish wastewater contained extensive coliform colonies, requiring serial dilutions for enumeration. Diverse coliform types, including those with colors suggestive of potential fecal contamination, highlighted the complexity and contamination risks inherent to aquaponic systems.

The analysis of commercial rocket leaves provided a standard for evaluating aquaponic produce. The commercial samples contained 4.8 g/100g dw of fat, 18.5 g/100g dw of ash, 36.2% crude protein, 35.6% fiber, and an energy content of 279.1 kcal/100g dw. Comparisons with literature suggest that aquaponically grown rocket may achieve similar or slightly enhanced nutrient profiles, benefiting from the continuous nutrient recycling in these systems.

These findings highlight the potential of aquaponic systems to produce high-quality, nutrient-dense crops while supporting a balanced microbial ecosystem. However, the significant microbial load in fish wastewater imposes strategic water treatment to control beneficial microbes while mitigating risks from pathogens and contaminants. Nutrient-rich aquaponic environments have the capacity to enhance plant growth and nutritional quality, offering a sustainable alternative to traditional farming methods. By integrating robust microbial management with optimized growing conditions, aquaponic systems can support sustainable food production while maintaining the health of fish, plants, and the overall

ecosystem. These insights contribute to refining aquaponic practices for improved productivity and ecological balance.

7. Perspectives futures

To further develop the decoupled aquaponic system and improve its overall performance, several important adjustments should be considered based on the results obtained so far. First, focusing on the water source is essential. It was observed fluctuations in pH and electrical conductivity (EC) in the previous experiments, which can significantly affect plant growth. Experimenting with different types of water, such as integrating more stabilized water treatments or adjusting the mineral composition of the aquaculture water, could help mitigate these fluctuations and provide a more balanced nutrient profile. In addition, fish species selection plays a critical role in nutrient supply. Another possibility is to test different species with higher nutrient excretion rates, or those more adaptable to the specific conditions of our system, to better match the nutritional needs of the plants. It's also worth considering the size of the fish relative to the plant demands; for example, a larger species might produce more waste, but may also require more space or oxygen, which could impact overall system balance.

Another factor to consider is germination time. Extending the germination period, as discussed, would give the seedlings more time to establish themselves and better adapt to the system's conditions before being exposed to the full nutrient load. This could lead to healthier, stronger plants with higher resistance to stress, thus improving overall system productivity.

In terms of plant species, while rocket plant was the initial focus, there's value in exploring a broader range of crops that might respond better to the decoupled aquaponic system. For example, testing with fast-growing leafy greens like lettuce, spinach, or herbs such as basil or mint could offer valuable data on how different plant types perform in such a system. Each species has its own nutrient and water needs, and optimizing the system for the right plants could lead to higher yields and more efficient nutrient cycling.

As the system performs more efficiently, scaling it up to include multiple grow beds or different fish tanks could allow for the simultaneous cultivation of a variety of plants. This would be a key consideration for commercial applications. Additionally, incorporating automated monitoring and control systems could improve the management of water

parameters like pH, temperature, and EC. Automated systems could also help with adjusting nutrient levels in real time, reducing the need for manual interventions and making the system more sustainable in the long run.

Finally, energy efficiency is another critical factor. Since aquaponic systems can sometimes be energy-intensive, particularly with air pumps, filtration systems, and grow lights, must be investigate renewable energy options like solar power or wind to reduce the carbon footprint. This would make the system more environmentally friendly and economically viable in the long run.

By exploring these areas and integrating additional technologies, it is possible to refine the decoupled aquaponic system to achieve a more sustainable, efficient, and scalable model for urban farming. This approach not only holds promise for improving plant yields but also provides a more adaptable and environmentally conscious solution for growing food in resource-limited settings.

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