POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Study of the potential of the macroalgae Halopteris scoparia in aquaculture production

Marcia Perestrelo Abreu

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Dissertation for obtaining the Master's Degree in Aquaculture

Master´s dissertation conducted under the supervision of Doctor Teresa Mouga and Doctor Ana Pombo

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Resumo

O presente estudo explorou as condições de cultivo e o crescimento de *Halopteris scoparia*, com o objetivo de otimizar o seu crescimento para a possível utilização em sistemas de Aquacultura Multi-Trófica Integrada (IMTA) em junção com peixes marinhos. Talos de *H. scoparia* saudáveis foram recolhidos e aclimatados com o intuito de avaliar os efeitos de diferentes meios de cultura, salinidades, densidades, luz, temperatura e o seu comportamento em águas residuais de uma aquacultura de peixe marinho. Ao longo dos ensaios, observou-se que estes fatores influenciaram significativamente, a Taxa de Crescimento (RGR) e Produtividade de *H. scoparia*. A introdução do meio de cultura Nutribloom® levou a um aumento significativo nas taxas de crescimento. A macroalga apresentou uma alta adaptabilidade a diferentes salinidades e um crescimento ótimo densidades mais baixas, preferencialmente 1g/L. A utilização de luz LED branca também melhorou o desempenho do crescimento. Os ensaios de temperatura não revelaram resultados conclusivos. *H. scoparia* demonstrou resultados promissores quando cultivada em águas residuais de uma piscicultura de corvina (*Argyrosomus regius*) em sistema de recirculação de aquacultura (RAS), tanto em forma concentrada como diluída. Por fim, a análise da biomassa cultivada revelou diferenças em termos de humidade e conteúdo de cinzas em comparação com a alga selvagem, destacando a influência das condições de cultivo na composição da alga. Em suma, o estudo destaca o potencial desta espécie para um crescimento rápido em aquacultura e para a sua integração em sistemas IMTA.

Palavras-chave: Macroalgas, Cultivo vegetativo, Águas residuais, Parâmetros físico-químicos.

Abstract

This thesis explored the cultivation conditions and growth patterns of *Halopteris scoparia* under controlled laboratory settings, with the goal of optimizing its growth for potential use in Integrated Multi-Trophic Aquaculture (IMTA) systems alongside marine fish. Healthy, wild thalli of *H. scoparia* were collected, acclimated, and subjected to various experiments to assess the effects of different culture media, salinity levels, stocking densities, light conditions, temperature, and wastewater reuse. Throughout the trials, it was observed that these factors significantly influenced the key growth parameters, Relative Growth Rate (RGR) and productivity, of *H. scoparia*. Notably, the introduction of the commercial medium Nutribloom® led to a significant increase in growth rates. The macroalgae presented adaptability to different salinities and an optimal growth with lower stocking densities (1g/L). The use of white LED light also enhanced growth performance, while temperature trials did not yield conclusive results. Furthermore, *H. scoparia* demonstrated positive growth when cultivated in wastewater from a meagre (*Argyrosomus regius*) fish farm using a recirculating aquaculture system (RAS), both in full-strength and diluted forms. Analysis of the cultivated biomass revealed notable differences in humidity and ash content compared to wild specimens, underscoring the influence of cultivation conditions on seaweed composition. Overall, the study highlights the species' potential for rapid growth in aquaculture and its suitability for integration into IMTA systems.

Key-Words: Macroalgae, Vegetative growth, Wastewater; proximate analysis.

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1 Introduction

1.1 Macroalgae

Macroalgae are large, multicellular, macroscopic, photosynthetic organisms. They are classified into three main groups, brown, green, and red algae, due mainly based on their pigment colour (Phaeophyceae, Chlorophyta and Rhodophyta, respectively). Known as seaweeds when exclusively marine, these organisms present a great variety of forms and functions and can offer a variety of uses starting from source of nutrition, cosmetics, fertilizer, medicinal among others (Gothandam et al., 2020).

Seaweeds play an important role in marine ecosystems as primary producers, providing an energy foundation for every aquatic organism, consequently being essential for the ecosystem's equilibrium (Farghali et al., 2023). Additionally, they offer several key benefits like providing a crucial food source to herbivorous fish and invertebrates, and cycling excess nutrients present in their environment. They are also recognized for being the foundation organism for a large number of habitats because due to their ability to create structured environments from simple surfaces to extremely elaborated ones, supporting numerous sessile and mobile species (Bruno & O'Connor, 2005; Navarro-Barranco et al., 2018; Thomsen et al., 2020).

Through photosynthesis, macroalgae fix carbon (Thomsen et al., 2020) and absorb nutrients from the seawater during their growth process, regardless of the nutrient concentration in the seawater. They also release oxygen supplementing the dissolved oxygen in the water, balance the pH of the seawater, and efficiently maintain the stability of the marine ecosystem. Although different algae species have different absorption rates on nitrogen (N) and phosphorus (P), cultivating macroalgae is nonetheless an extra high-quality bioremediation measure for seawater eutrophication (Ye et al., 2023; Zhang et al., 2019).

1.2 Phaeophyceae

Phaeophyceae are found almost exclusively in marine habitats and play a crucial role in the benthic vegetation of rocky shores in the northern and southern hemispheres. Their colour, which varies from yellow to black, is due to the presence of the xanthophyll pigment fucoxanthin, which dominates over other pigments such as chlorophylls *a*, *c1* and *c2* (Pereira & Neto, 2015). The class Phaeophyceae is estimated to have around 285 genera and 2129 species (Guiry & Guiry, 2024), being from freshwater habitats less than 1% (Wehr et al., 2015).

Brown macroalgae present a viable option for aquaculture due to their remarkable biomass productivity, allowing multiple harvests along the year and their high photosynthesis efficiency resulting in a remarkable biomass productivity (Chen et al., 2015; Lee et al., 2024; Sarwer et al., 2022).

1.3 *Halopteris scoparia*

1.3.1 Classification

Table 1-Taxonomic classification of *Halopteris scoparia* (source:(Guiry & Guiry, 2024))

Empire	Eukaryota
Kingdom	Chromista
Phylum	Heterokontophyta
Subphylum	Ochrophytina
Class	Phaeophyceae
Subclass	Dictyotophycidae
Order	Sphacelariales
Family	Stypocaulaceae
Genus	*Halopteris*

Halopteris scoparia (Linnaeus) Sauvageau 1904, previously known as *Stypocaulon scoparium* (Draisma et al., 2010; Patarra et al., 2017), is a dark brown alga that forms fluffy clumps in shallow waters with rocky bottoms. Growing up to 15 cm in length, *H. scoparia* has a main axis with alternating branches and fan-like shape when flat, and forms inverted cone-shaped tufts when suspended in the water column. The algae are normally attached to rocks with small to large disks, which are often hidden by many matted rhizoids (Pereira, 2016). This species is distributed in the Atlantic Ocean and can be found throughout the year in mainland Portugal, Madeira, Azores, Canary Islands (Patarra et al., 2017), in the Mediterranean, Cape Verde (Silva, 2009), Black Sea and Adriatic Sea (Pereira, 2016).

Figure 1- *Halopteris scoparia* specimen under a magnifier, captured using 5 x magnification on a stereomicroscope.

1.4 Epiphytes

Epiphytism is prevalent in marine benthic communities, were epiphytes (multicellular algae that live on other photosynthetic organisms), attaching themselves to the host algae or seagrass (Jover et al., 2021). In many ecological communities, epiphytes are considered an important component that can provide additional carbon sequestration, biomass and niche variability (Lobelle et al., 2013). However, they can also compete with the host algae interfering in nutrient absorption, light capture, carbon uptake and biomass production of the seaweed (Pang et al., 2011, 2022), reducing the production of host algae (Hurtado et al., 2006; Pang et al., 2015, 2022; Vairappan et al., 2008).

Epiphyte infestations can also have a major impact in cultivated macroalgae, resulting in reduce growth rates and reduced yields, attributed to the competitive pressure for resources (Usandizaga et al., 2023). In the 1970s, the first recorded epiphytes outbreaks caught the attention of several researchers, which causes slower growth and notable biomass loss of the cultivated macroalgae, leading to a low-quality product (Gothandam et al., 2020).

1.5 Macroalgae cultivation

The cultivation of macroalgae has gained recognition for its potential to contribute to sustainable goals such as improving food security, restoring ecosystems, and mitigating climate change (Duarte et al., 2021). The production of macroalgae in Europe is a small but an emerging industry (Araújo et al., 2021; Camarena-Gómez et al., 2022), depending largely on the harvesting of wild seaweed (Cerca et al., 2023). This industry serves various applications such as food and feed, cosmetics, pharmaceuticals, biofuels among other areas (Cerca et al., 2023; Kraan, 2020).

In 2022, the global algae production increased 1.4 million tonnes when compared to 2020, reaching 36.5 million tonnes of farmed algae (FAO, 2024). Aquaculture seaweed production supplies the greater share of macroalga, having 97% of the global

macroalgae production in 2019 (FAO & WHO, 2022), representing nearly 30% of the world's aquaculture (FAO, 2021). Mostly of the seaweed aquaculture occur in Asian countries (China, Korea and Japan) (Redmond et al., 2014), with 97.3% of the global production, followed by America (1.39%) and Europe (0.80%) (FAO & WHO, 2022). The brown seaweed production has increased from 13000 tonnes in 1950 to 16.4 million tonnes in 2019, being *Laminaria/Saccharina* and *Undaria* the most cultivated genera (FAO, 2021).

Macroalgae production industries have the capacity to produce massive amounts of nutrient-rich food for human consumption with a lower environmental impact compared to land-based agriculture (Tiwari & Troy, 2015). Macroalgae can absorb eutrophicating pollutants, such as nitrogen (N) and phosphorus (P) (Mhatre-Naik et al., 2021). They also have the capacity to remove metal contaminants from saline waters (Henriques et al., 2015, 2017, 2019; Jacinto et al., 2018; Pinto et al., 2020; Smii et al., 2023). Thus, they provide a versatile and sustainable way to remove excess nutrients from both freshwater and marine waste streams to meet environmental standards (Praeger et al., 2020). This addresses the increasing concern on environmental sustainability due to the eutrophication and ecological imbalance in coastal areas. One of the main sources of nutrient enrichment in coastal waters is aquaculture, right next to domestic waste (Aníbal et al., 2014; Marinho-Soriano et al., 2009).

Macroalgae aquaculture can be conducted in land-based tanks, being mostly done in open water like ponds, offshore or on coastal waters using different layouts of nets, ropes, or rafts (Araújo et al., 2021; Camarena-Gómez et al., 2022). The growth of seaweeds is controlled by abiotic and biotic factors (Kotta et al., 2022), being light, temperature, salinity, water motion and nutrient availability the major environmental factors that influence their growth (Hurd et al., 2014).

The availability of light is influenced by the amount of irradiance received at the sea surface, influencing the photosynthetic rates of the seaweed (Anthony et al., 2004; Kotta et al., 2022), and self-shading within their habitat. Self-shading can become a critical limitation in natural algal assembles limiting the amount of photosynthesis that

the macroalgae can do (Kotta et al., 2022; Tait & Schiel, 2010). Light can also cause oxidative damage, for that reason macroalgae, and other photosynthetic organisms need to regulate light intake to be able to prevent that type of damage (Xie et al., 2020). In land-based aquaculture, light can be a highly dynamic variable influenced by internal and external factors. Different light regimes impact the physiology of cultured macroalgae affecting algal growth rate, bioactive molecule concentration and epiphyte overgrowth dynamics (Marques et al., 2022).

The use of different wavelengths (Fig. 2) can also affect the growth of the algae since the different pigments can perform distinctly. Chlorophyll *a* shows a higher light absorption in blue and red wave lengths, while carotenoids can enhance absorption in blue wave lengths (Thoral et al., 2023).

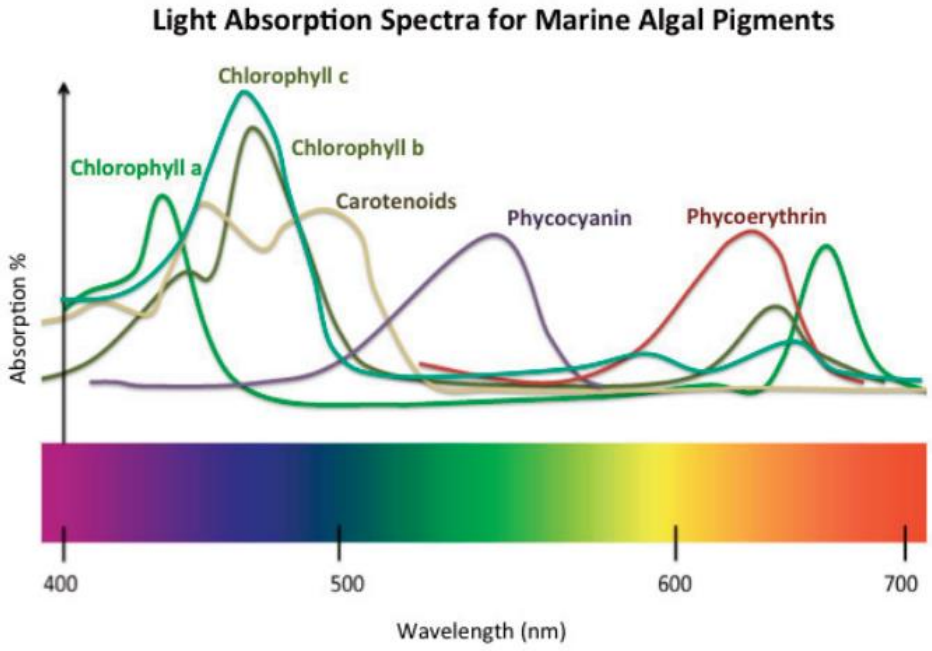


Figure 2- Light Absorption Spectra for Marine Algal Pigments; Source: New England Seaweed Culture Handbook

Intertidal algae are affected with the course of tidal cycles, which causes them to be emersed for certain time periods and need to develop strategies to survive to these harsh conditions, showing a high stress tolerance ability (Zheng et al., 2019). These

algae have the ability to control the amount of light harvested, to prevent damage (Xie et al., 2020).

The availability of nutrients highly affects the macroalgal growth (Kotta et al., 2022; Raven & Hurd, 2012), seaweeds present better growth rates in areas where high levels of phosphorus and nitrogen are found (Kotta et al., 2022). That way macroalgae can provide multiple environmental benefits, including eutrophication mitigation, carbon capture among others (Farghali et al., 2022). In fish production only about 30% of the N and P in the fish feed turns into muscle, around 70% ends up being released into the water (Hurd et al., 2014). Thus, combining fish culture with the culture of other low-trophic level species that use waste products can minimize waste and increase production efficiency (Kleitou et al., 2018). Therefore, the production of seaweed can be combined with other types of aquacultures, fish and/or mollusc farming, in Integrated Multitrophic Aquaculture (IMTA), where its cultivation can compensate for the excess of nutrients that are released by fish farming (Camarena-Gómez et al., 2022). This combination, when paired with species of great economical value, can provide an extra income to the producer (Mhatre-Naik et al., 2021).

The production of macroalgae biomass can bring several socioeconomics and environmental benefits (Camarena-Gómez et al., 2022), and can also provide, when correctly imposed, a sustainable circular bioeconomy strategy (Barbier et al., 2020; Farghali et al., 2022)

1.6 Integrated Multi-trophic Aquaculture

Aquaculture has increased significantly due to the demand for animal protein (Ye et al., 2023) and, consequently, an increasing demand for aquatic resources. To meet that demand, there was a need to intensify the aquaculture systems, raising concerns about the pollution caused by these systems (Hughes & King, 2023).

In an effort to create more ecological friendly systems, IMTA (Integrated Multi-trophic Aquaculture) systems were introduced in the 1970's in USA as pilot projects,

mimicking the structures and functions of natural environments, reusing waste practicing nutrient recycling (D'Orbcastel et al., 2022). This allows the recapture of uneaten feed, dissolved nutrients, and by-product particulate wastes by other species to be converted to energy, feed or fertilizer (Kleitou et al., 2018; Neori et al., 2000; Troell et al., 2003; Zhang et al., 2009). These systems also have another advantage, which is that they make it possible to increase the number of species produced, increasing production diversification (Califano et al., 2020). This type of system is highly recognised for its sustainability as it can promote ecological efficiency, environmental acceptability, diversity in products, profitability, and social benefits (Kleitou et al., 2018).

Macroalgae nourished by effluents use photosynthesis to grow reintroducing energy into the system (D'Orbcastel et al., 2022), enriching seawater with oxygen and naturally mitigate the excess dissolved carbon dioxide and nutrients present in their surrounding (Giangrande et al., 2020).

1.7 Objectives

The aim of this study was to determine if *Halopteris scoparia* was a suitable specie to produce in aquaculture and to identify the optimal conditions for its growth. The study aimed to answer the following questions:

- Is it possible to produce *Halopteris scoparia* in aquaculture settings?
- What are the optimal growth conditions, including nutrients, density, salinity, temperature, and light to produce this species?
- Can *H. scoparia* be cultivated in wastewater from marine fish farming?
- Do different cultivation conditions affect ash and moisture content of the seaweed?

2 Methods

Throughout the study there were conducted several experiments to allow us to understand the ideal growth conditions, and the influence of different abiotic factors on *Halopteris scoparia*, which included nutrients, salinity (psu), light irradiance (µmol $m^{-2}s^{-1}$), density (g/L), and temperature (ºC). It was also analysed the growth of the algae with wastewater from SEAentia, an aquaculture of Meagre (*Argyrosomus regius* (Asso, 1801)). Lastly, it was also analysed the moisture and ash contents of the wild and cultivated alga.

2.1 Sampling and acclimatization

The wild *Halopteris scoparia* was collected, in “Praia do Quebrado”, in Peniche, Portugal (39º 36.713’ N, 9º 37.405’), a rocky area that is usually covered by water during the hight tides. After the collection of the specimens, these were cleaned and selected, removing all the debris, unwanted organisms, and necrotic tips. Afterwards, the macroalga was submerged in filtrated freshwater, for 30 seconds, to further eliminate unwanted organisms.

The following week, the alga was kept in laboratory trays with constant aeration in a controlled temperature room (19 ± 1ºC), with White LED (Photosynthetic Photon Flux Density of 61±2 µmol $m^{-2}s^{-1}$), 16h/8h photoperiod, and a salinity of 35 ± 2 psu for acclimatization purposes (Freitas et al., 2021).

2.2 Trial preparation

For the nutrients, salinity, light, and density trials, a smaller volume (1L) was used in a flat-bottom flask. In these trials all the material, filtered seawater and Von Stoch Enriched (VSE) medium was sterilized, using an autoclave (105 ºC, for 20 minutes). The seawater used was first diluted with filtered freshwater, to the desired salinity (25, 30 or 35 ± 1 psu) then filtered through a 0.2 µm filter to remove any

unwanted debris and organisms. Seawater was then stored in glass containers (Schott) for sterilisation.

For the trials of temperature and IMTA, bigger volumes were used, 5 L flat-bottom flasks. Due to its volume, instead of sterilization, the flasks were disinfected with sodium hypochlorite. The seawater did not undergo any sterilization cycle either, being simply diluted to the desired salinity and filtered through a 0.2 µm filter.

2.3 Nutrient media

There were two types of nutrient media used: VSE (Von Stosch Enriched) (Table 3), commonly used for macroalgae culture, which was previously prepared following Yarish *et al.* 2014. (Table 3), and Nutribloom® (Necton, Olhão, Portugal) (Table 4) a premade commercially available medium typically used for microalgae. Both media were stored at 4 ± 1ºC.These media differ from each other in terms of nutrients and minerals and may provide different benefits to the macroalgae. Both nutrient media were tested, and it was verified that *H. scoparia* presented a better performance when using Nutribloom® as nutrient media. Consequently, Nutribloom® was selected as the preferred nutrient medium for the remaining trails.

Table 2- Quantities added of Nutribloom® and VSE (Von Stosch Enriched) per litter of seawater

Nutrient media		**Quantity**
Nutribloom®		1 ml/L
VSE (Von Stosch Enriched)	**Nitrogen**	1 ml/L
	Phosphate	1 ml/L
	Iron-EDTA	2 ml/L
	Manganese	1 ml/L
	Vitamins	0.025 ml/L

Table 3- Composition of the Von Stoch Enriched Medium according to Yarish et al. 2014

	VSE (Von Stosch Enriched)
Components	NH_4Cl
	$Na_2HPO_4.2H_2O$
	$FeSO_4.7H_2O$
	$Na_2EDTA.2H_2O$
	$MnCl_2.4H_2O$
	Thiamine
	Biotin
	Vitamin B12

Table 4 - Composition of the nutrient medium Nutribloom®

	Nutribloom®
Components	$NaNO_3$
	KH_2PO_4
	$ZnCl_2$
	$ZnSO_4$
	$MnCl_2$
	$Na_2MoO_4.2H_2O$
	$CoCl_2.6H_2O$
	$CuSO_4.5H_2O$
	EDTA
	$MgSO_4$
	$FeCl_3$
	Thiamine
	Biotin
	Vitamin B12

2.4 Light conditions

For most of the trials conducted (nutrient, salinity, light, density, and seawater from meagre aquaculture), the same light conditions (Table 6) were maintained to ensure consistency in experimental parameters. However, in the Temperature trial, the experimental setup differed due to limitations in the available equipment. Specifically, there were fewer lightbulbs available for this trial compared to the others, resulting in a lower light intensity (20 µmol m^{-2} s^{-1}). This discrepancy in light conditions may have affected the growth responses observed in the Temperature trial compared to the other trials, and this factor should be taken into consideration when interpreting the results.

White LED light (Glass LED 24W, 230Vac, 50/60Hz, 116mA), was used for all the trials and the red and Blue LED were used for the light trial.

Table 5- Parameters of the LED light used Photosynthetic Photon Flux Density (PPFD)

	PPFD (µmol.m^{-2}. s^{-1})	**(λP) (nm)**
White LED	60.45	450+550-620
Blue LED	63.29	455
Blue LED	63.43	657

2.5 Nutrient, salinity, light and density trials

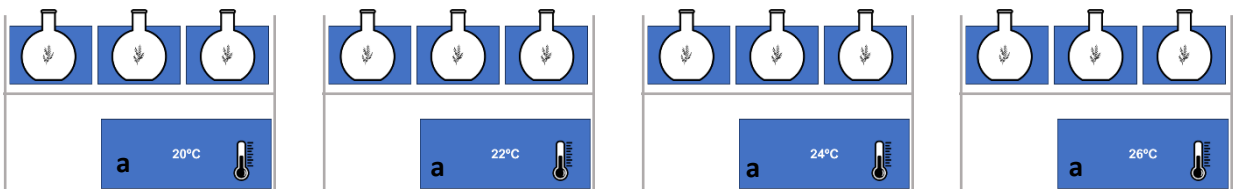
After a week of acclimatization, healthy tips with 1 cm length were selected and placed into 1 L flat bottom flasks, to the density of 1g/L, along with sterilized seawater enriched with nutrient media, in a controlled temperature room (19 ± 1ºC). The trials for the nutrients, salinity, light and density were run in similar conditions, only changing the factor in study. Details of the trials carried out can be found in Table 5.

Table 6- Conditions for the Nutrient, Salinity, Light and Density trials**.**

	Nutrient Media	Salinity (psu)	Density (g/L)	Light	Duration (weeks)
Nutrient	VSE Nutribloom	35	1	White LED	4
Salinity	Nutribloom	25 30 35	1	White LED	2
Light	Nutribloom	30	1	White LED Red LED Blue LED	2
Density	Nutribloom	30	1 2 3	White LED	2

2.6 Temperature trial

This experiment was conducted using 4 recirculating aquaculture systems (RAS) with 3 aquaria and one sump each. The flat-bottom flasks (6L) housing the algae were submerged in water baths set to the desired temperatures of 20, 22, 24, and 26 ºC. These temperatures were maintained by heaters with thermostats in the SUMP of each RAS system. Additionally, LED lights were also placed behind the tanks to provide light for the algae's growth. The trial was performed in triplicate and lasted for 4 weeks.



2.7 Trial with wastewater from a meagre aquaculture

The trial with wastewater from meagre aquaculture (MWT) was conducted to observe if the algae would survive and thrive in a IMTA environment. Wastewater of meagre rearing from a local company (SEAentia, Peniche, Portugal) that uses a RAS system was collected every week. The water was transported in 25 L plastic containers during 5 minutes to the Aquaculture Laboratory of MARE Polytechnic of Leiria. When in the laboratory the water, when not immediately used, was kept at 4 ± 1 ºC.

Three different concentrations of the meagre rearing wastewater (MW) were tested, 100%, 50% and 0%, in triplicate, over a period of 4 weeks, using 6L flat bottom flasks, in temperature-controlled room (19 ± 1ºC). Where there was no wastewater (0%), Nutribloom® was used as a nutrient medium. The nitrate initial concentration of nitrogen, previously tested by the company, in the meagre wastewater was of 77.3, 41.0, 64.5 and 35.0 mg/L, for week 1, 2, 3 and 4, respectively.

2.8 Data collection

2.8.1 Growth trials

In all the trials, the algae biomass was weighted on a weekly, providing a systematic assessment of their growth throughout the experiment. Simultaneously, the algae media was replaced weekly to ensure optimal nutrient conditions for the algae. The relative growth rate and productivity (RGR) were determined using the following formula (Patarra et al. 2017):

$$RGR(\%\ fw\ day^{-1}) = \frac{Ln(fw) - Ln(iw)}{t} * 100$$

iw- initial fresh weight (g)

fw- final fresh weight (g)

t- time (days)

$$Productivity\ (g\ dw\ m^{-2}day^{-1}) = 0.2723 * [((fw - iw)/t)/V]$$

fw-final fresh weight

iw- initial fresh weight

t- time(days)

V- Volume of each flask used

The value of 0.2723, stands for the proportion of dry to fresh weight of *H.* scoparia (based on fresh and dry weight of 43 samples).

2.8.2 Ash and Moisture

To determine the amount of moisture and ash present in the algae, 1g of biomass, from each trial and wild *H. scoparia*, was weighted in a crucible. The crucibles, containing the biomass, were placed in a laboratory oven at 60 ºC for 48h and then placed in a desiccator, once reached room temperature, the samples were, once again weighted. The humidity present in the algae was calculated using the following formula (AOAC, 2016):

$$Humidity\ (\%) = \frac{Wf - Wd}{Wf} * 100$$

W_f - Fresh sample weight (g)

W_d - Dried sample weight (g)

The samples collected from each trial, and wild *H. scoparia* were placed into to a muffle furnace (B170, Nabertherm, Germany) with a heating ramp of 4 hours a plateau at 525 ºC for 5 hours. The crucibles were removed after the oven cooled down and placed in a desiccator until they reached room temperature. Once they reached room

temperature, each crucible was weighted, and the ashes were calculated using the following formula (AOAC, 2016):

$$Ash(\%\ dry\ weight) = \frac{Wa}{Wd} * 100$$

W_a- Ash Weight (g)

W_d- Dried sample weight (g)

2.9 Statistical analysis

All the statistical analysis were performed using the software IBM SPSS 28.0 (IBM Corporation, Armonk, New York, U.S). The results were expressed as mean standard deviation (SD) and *p*-value < 0.05 was the value chosen for significance. In order to access if the data meet the assumptions of analyses of variance (ANOVA), it was tested for normality with the Levene's test and for homogeneity with Shapiro-Wilk test. Whenever the assumptions were not met, the Kruskal-Wallis test was performed.

3 Results

3.1 Growth trials

3.1.1 Nutrient trial

For this experiment the Relative Growth Rate (RGR) (Figure 3) was tested for two different types of nutrient media, VSE and Nutribloom®, it was observed higher growth rate with Nutribloom® for the 4 weeks of trial. *H. scoparia* presented a higher RGR value with Nutribloom® on Week 3 (10.49 ± 1.07% fw day^{-1}) and with VSE it was also observed a higher RGR value on Week 3 (4.71 ± 1.77 % fw day^{-1}). In terms of statistical analysis, all the assumptions were meet for Week 2, 3 and 4 but only Week 2 ($F_{(1)}$ = 28.901; *p*-value= 0.006) and 3 ($F_{(1)}$ = 17.021; *p*-value= 0.015) showed statistically significant differences in the growth of *H. scoparia*, while in Week 4 ($F_{(1)}$= 0.599; *p*-value= 0.482) a *p*-value> 0.05 was observed.

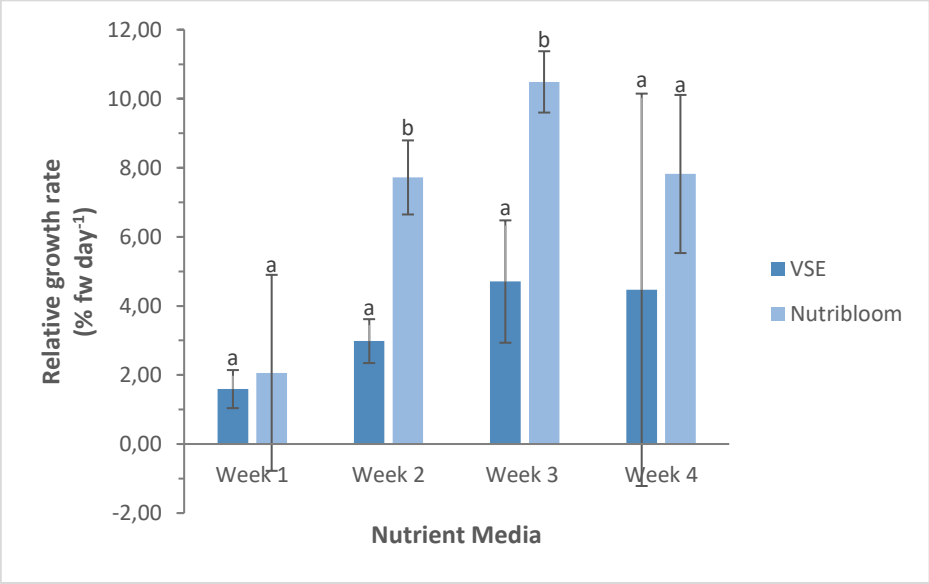


Figure 3- Relative growth rate (% fw day^{-1}) (Mean ± SD) of *Halopteris scoparia* with two different nutrient media, VSE (Von-Stosch Enriched) and Nutribloom®. Different letters above the bars indicate statistical differences for the Relative Growth Rate between treatments for each week (ANOVA, *p*-value < 0.05).

In terms of productivity (Figure 4), we can see that Nutribloom® reached a higher productivity value throughout the experiment, reaching its highest value of 40.85±15.31 g dw $m^{-3}day^{-1}$in Week 4. VSE presented the lowest values during the experiment when compared to Nutribloom®. All the assumptions were met for Week 2, 3 and 4, therefore an ANOVA was performed. There were significant differences for Week 2 ($F(1)=17.861$; *p*-value= 0.013) and Week 3 ($F(1)=22.739$; *p*-value = 0.09), while Week 1 ($F(1)=0.043$; *p*-value = 0.853) and Week 4 ($F(1)=1.713$; *p*-value = 0.261) presented a *p*-value >0.05.

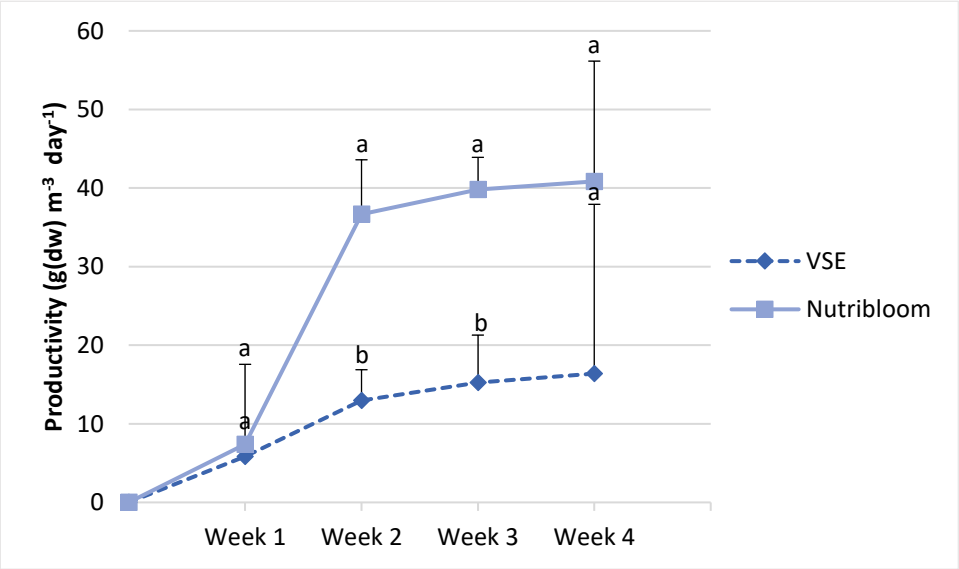


Figure 4- Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout 4 weeks, with two different media, VSE and Nutribloom®. Letters above the mean values indicate statistical differences for the productivity between treatments for each week (ANOVA, *p*-value < 0.05)

3.1.2 Salinity trial

In terms of salinity, it was observed that the RGR (Figure 5) was higher in Week 2 for all the salinity levels when compared to Week 1. In Week 1, RGR was higher at a salinity of 25 psu (5.08 ± 0.75% fw day-1) and lower at 30 psu (1.89 ± 0.45% fw day-

^{1}). Week 2 showed a higher RGR in 30 psu (10.47± 0.74% fw day^{-1}) and lower RGR in 25 psu (7.21± 1.75% fw day^{-1}). For the statistical analysis no significant statistical differences were found for Week 1 ($F_{(2)}$=5.600; *p*-value= 0.061) and Week 2 ($F_{(2)}$= 2.173; *p*-value= 0.195).

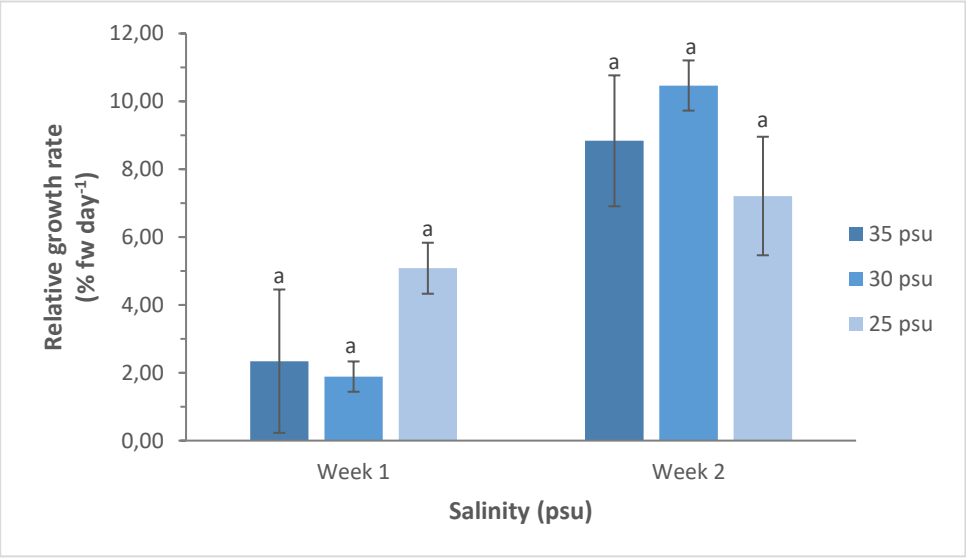


Figure 5- Relative growth rate of *Halopteris scoparia* (% fw day^{-1}) (Mean ± SD) with three different salinities, 35, 30 and 25 psu. Different letters above bars indicate statistical differences for the Relative Growth Rate between treatments for each week (ANOVA; *p*-value < 0.05)

For the productivity (Figure 6), we can observe that 30 psu presents a higher productivity increase, and the higher productivity value (50.17±7.46 g dw m^{-3}day^{-1}). Week 1 presented the highest value at 25 psu (17.51± 3.28) and lowest at 30 psu (5.72± 1.46). For week 2 you can observe that salinities 35 and 30 have similar values, 38.69± 5.41 g dw m^{-3}day^{-1} and 38.80± 11.39 g dw m^{-3}day^{-1}, respectively. There were no statistical differences for Week 1($F_{(2)}$= 4.327; *p*-value= 0.069) and Week 2($F_{(2)}$= 1.244; *p*- value=0.353). All the assumptions were met.

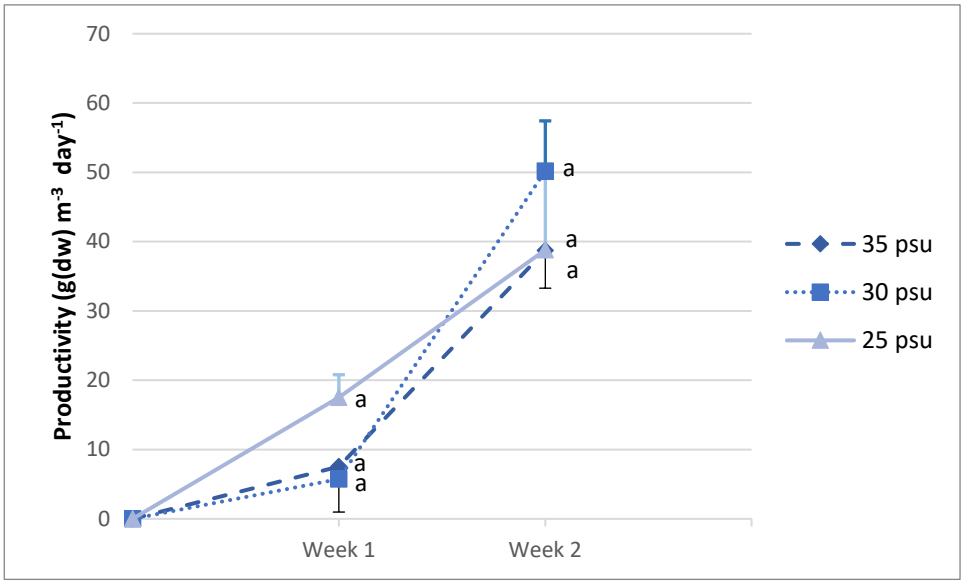


Figure 6 Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout 2 weeks, with three different salinities, 25, 30 and 35 psu. Different letters above the mean values indicate statistical differences for the productivity between treatments in each week (ANOVA, *p*-value < 0.05).

3.1.3 Density trial

Halopteris scoparia presented the highest relative growth rate (Figure 7) in the density 1g/L with 5.23 ± 1.43% fw day^{-1}(Week 1) and 8.46 ± 1.57% fw day^{-1}(Week 2). The lowest RGR was recorded with the density of 3g/L with a mean percentage of 0.13 ± 0.48% fw day^{-1} and 5.03 ± 1.13% fw day^{-1} for Week 1 and Week 2, respectively. All the assumption for normality and homogeneity were met, therefore an ANOVA was performed. The statistical analysis showed that Week 1 ($F_{(2)}$ =14.833; *p*-value=0.005) showed statistically significant differences, while Week 2 ($F_{(2)}$= 4.991; *p*-value= 0.053) presented a *p*-value>0.05 with no statistically significant differences in growth between densities.

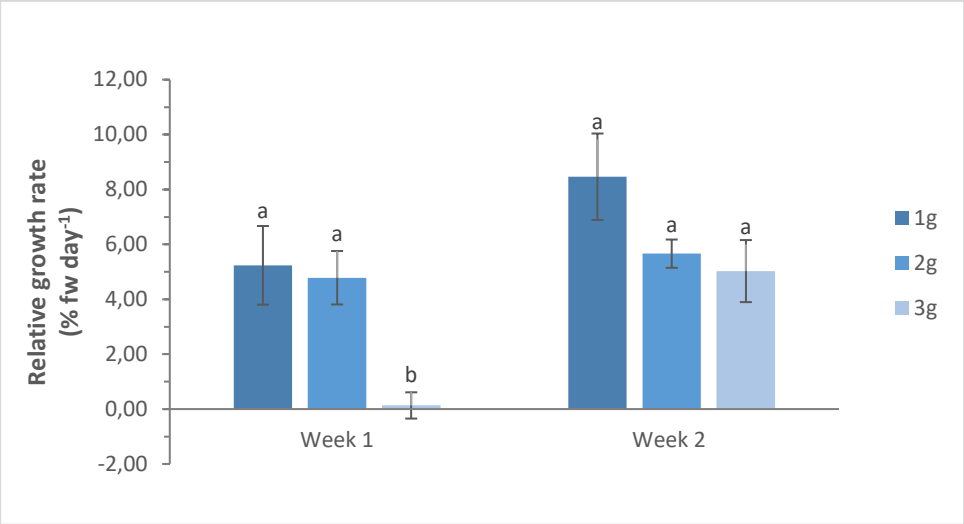


Figure 7- Relative Growth Rate (RGR) of *Halopteris scoparia* (% fw day^{-1}) (Mean± SD) with 3 different densities 1g/L, 2g/L and 3g/L. Different letters above bars the indicate statistical differences for the Relative Growth Rate between treatments for each week (ANOVA; *p*-value < 0.05)

In Figure 8, when can observe the productivity for the density assay. Density 2g/L presented a higher productivity value for Week 1 (31.30± 7.38 g dw $m^{-3}day^{-1}$) and Week 2 (53.27± 6.88 g dw $m^{-3}day^{-1}$). The lowest productivity value was registered for 3g/L in Week 1 (1.15± 3.94 g dw $m^{-3}day^{-1}$). Density 3g/L also presented the highest increase between weeks 1 and 2. Even though density 1g/L presented the highest RGR throughout the experiment, it presented the lowest productivity in Week 2 (46.72± 13.16 g dw $m^{-3}day^{-1}$). The statistical analysis showed statistical differences between densities for Week 1 ($F_{(2)}$= 13.504; *p*-value =0.006), while Week 2 ($F_{(2)}$= 0.177; *p*-value =0.842) presented no statistical differences. All the assumptions were met.

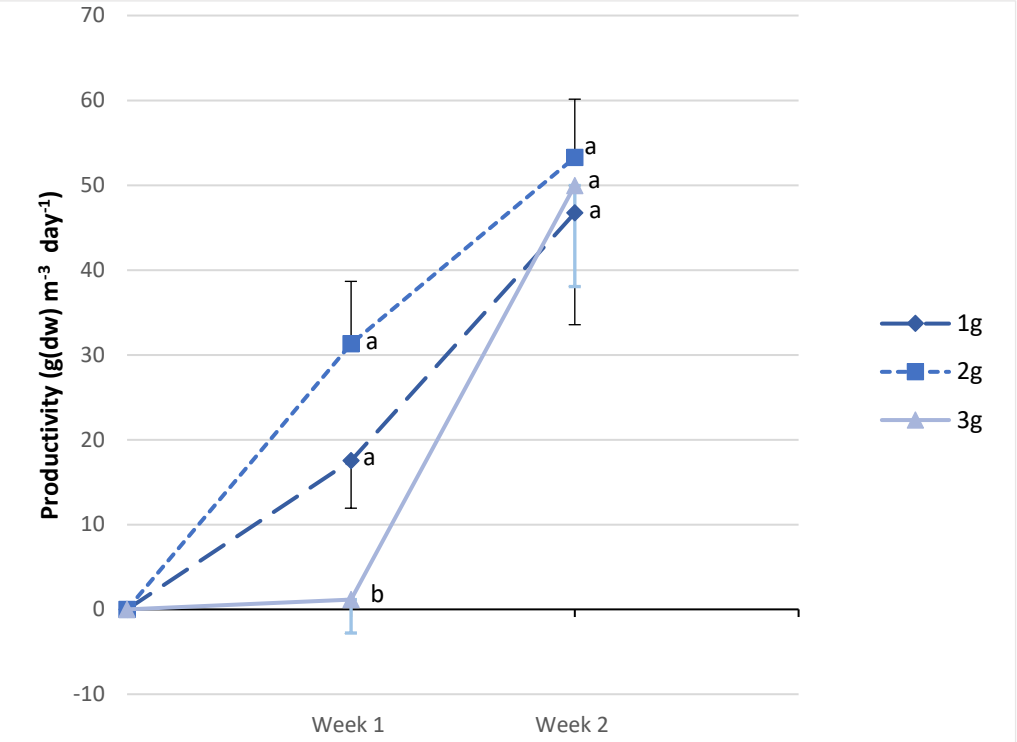


Figure 8- Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout 2 weeks, with three different densities, 1, 2, and 3g/L. Different letters above the mean values indicate statistical differences for the productivity between densities in each week (ANOVA, *p*-value < 0.05).

3.1.4 Light trial

In the light trial, for the White LED light, *H. scoparia* presented a RGR (Figure 9) with percentages of 3.49 ± 0.64% fw day^{-1} and 7.22 ± 1.52% fw day^{-1}, for Week 1 and Week 2, respectively. Algae under the Red LED light displayed a negative RGR in Week 1 (-0.09 ± 0.09% fw day^{-1}). The Blue LED light presented a RGR of 0.82 ± 1.63% fw day^{-1}, 2.97 ±1.15% fw day^{-1} and 1.90 ± 0.52 for Week 1 and Week 2, respectively. Every assumption was met for this trial, and only Week 1 ($F_{(2)}$=7.310; *p*-value=0.025) showed statistically significant differences.

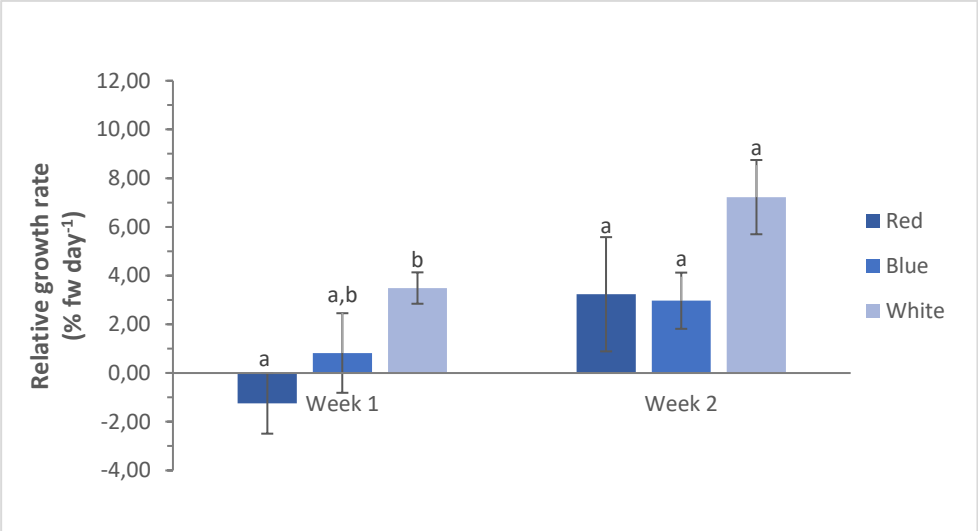


Figure 9- Relative Growth Rate (RGR) of *Halopteris scoparia* (% fw day-1) (Mean ± SD) under the effect of 3 different lights Red LED, Blue LED, White LED. Different letters above bars indicate statistical differences for the Relative Growth Rate between treatments in each week (ANOVA, *p*-value < 0.05)

For productivity (Figure 10), White LED showed the highest productivity values for Week 1 (10.66± 2.40 g dw $m^{-3}day^{-1}$) and Week 2 (32.25± 7.57 g dw $m^{-3}day^{-1}$). In contrast, Red and Blue LED displayed similar productivity values for Week 2 (10.05± 7.98 g dw $m^{-3}day^{-1}$and 9.80± 2.24 g dw $m^{-3}day^{-1}$, respectively). Red LED presented the lowest productivity for Week 1(-3.49± 3.56 g dw $m^{-3}day^{-1}$). All the assumption for these statistical tests were met, hence an ANOVA was performed. Statistical analysis revealed significant differences in productivity in Week 1 ($F_{(2)}$= 6.543; *p*-value= 0.031) and Week 2 ($F_{(2)}$ =7.907; *p*-value=0.021)

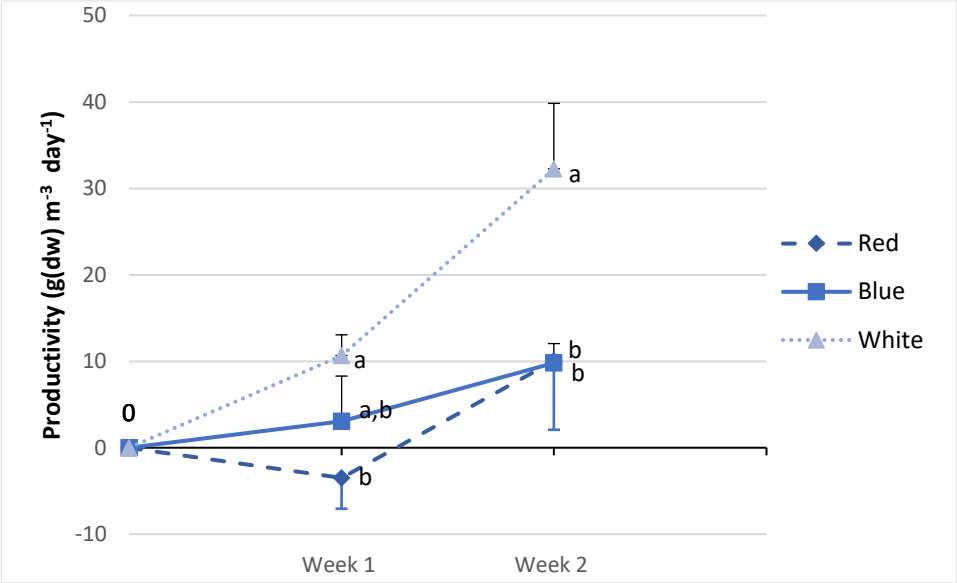


Figure 10- Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout 2 weeks, with tree different lights, Red LED, Blue LED and White LED. Different letters near the mean values indicate statistical differences for the productivity between treatments in each week (ANOVA, *p*-value < 0.05).

3.1.5 Temperature trial

For the temperature trial, it was mostly observed a decrease in RGR (Figure 11) during the experiment, with the biggest decrease in Week 2 with the temperature of 26 ºC, which registered a negative mean growth of -3.01±1.06% fw day^{-1}. The lowest RGR registered in Week 1 (-0.58 ± 0.48% fw day^{-1}) and Week 4 (-1.71 ± 1.24% fw day^{-1}) were obtained with a temperature of 24 ºC, for Week 2 (-3.01 ± 1.06% fw day^{-1}) 26ºC and, lastly, for Week 3 (-0.73 ± 1.68% fw day^{-1}) with the temperature of 22ºC. The highest RGR for the duration of the trial were obtained at 20ºC in Week 1 and Week 3, with a positive growth of 1.12 ± 0.36% fw day^{-1} and 0.44 ± 0.61% fw day^{-1}, respectively. There were no significant statistical differences between temperatures assayed for Week 1($F_{(3)}$= 1.650; *p*-value= 0.254), Week 2 ($F_{(3)}$=0.530; *p*-value=0.674), Week 3 ($F_{(3)}$=1.154; *p*-value= 0.764) nor for Week 4 ($F_{(3)}$= 0.296; *p*-value= 0.827).

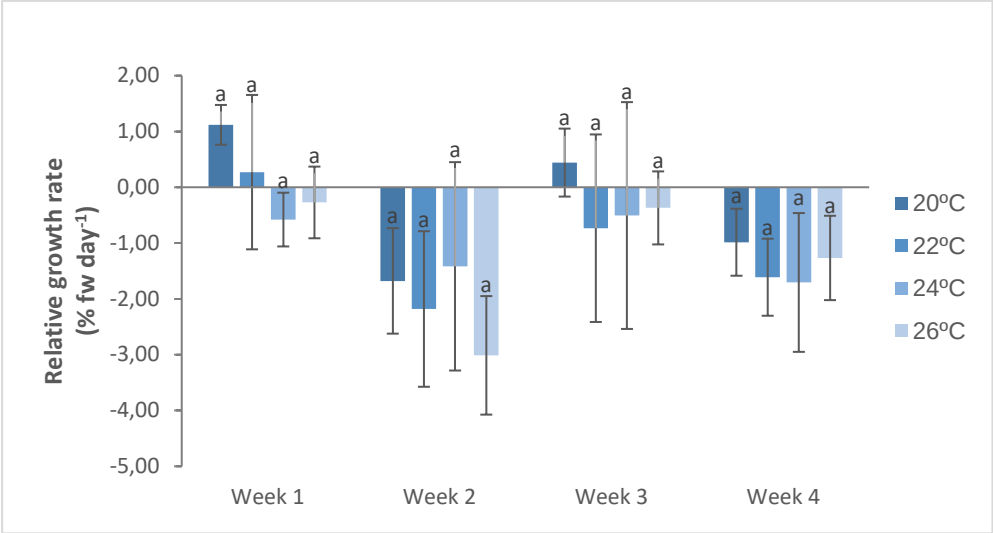


Figure 11- Relative Growth Rate (RGR) (% fw day^{-1}) (Mean ± SD) on *Halopteris scoparia* for four different temperatures 20ºC, 22ºC, 24ºC, 26ºC. Different letters above bars indicate statistical differences for the Relative Growth Rate between treatments for each week (ANOVA, *p*-value < 0.05).

For productivity (Figure 12), only two temperatures showed positive values in week 1, 20 ºC (3.45± 1.15 g dw m^{-3}day^{-1}) and 22ºC (0.98± 4.20 g dw m^{-3}day^{-1}), while 24ºC (-1.66± 1.36 g dw m^{-3}day^{-1}) showed the lowest value. In Week 2, a decrease in productivity was observed, with all values being negative, the highest at 24ºC (-3.46± 4.86 g dw m^{-3}day^{-1}), and the lowest at 26ºC (-7.87± 2.69 g dw m^{-3}day^{-1}). Week 3 showed an increase in productivity, with the highest productivity value recorded at 20ºC (1.23± 1.78 g dw m^{-3}day^{-1}) and the lowest at 22ºC (-1.71± 4.05 g dw m^{-3}day^{-1}). In Week 4, there was decrease in all temperatures, with 20ºC (-2.80± 1.70 g dw m^{-3}day^{-1}) and 26ºC (-2.79± 1.70 g dw m^{-3}day^{-1}) presenting the highest productivity values, and 24ºC (-3.56 ± 2.72 g dw m^{-3}day^{-1}) the lowest. In terms of statistical analysis, there were no statistical difference found for Week 1 ($F_{(3)}$=1.668; *p*-value= 0.250), Week 2 ($F_{(3)}$=0.501; *p*-value= 0.692), Week 3 ($F_{(3)}$= 0.455; *p*-value= 0.721) or Week 4 ($F_{(3)}$=0.163; *p*-value=0.918). All the assumptions for the statistical test were met.

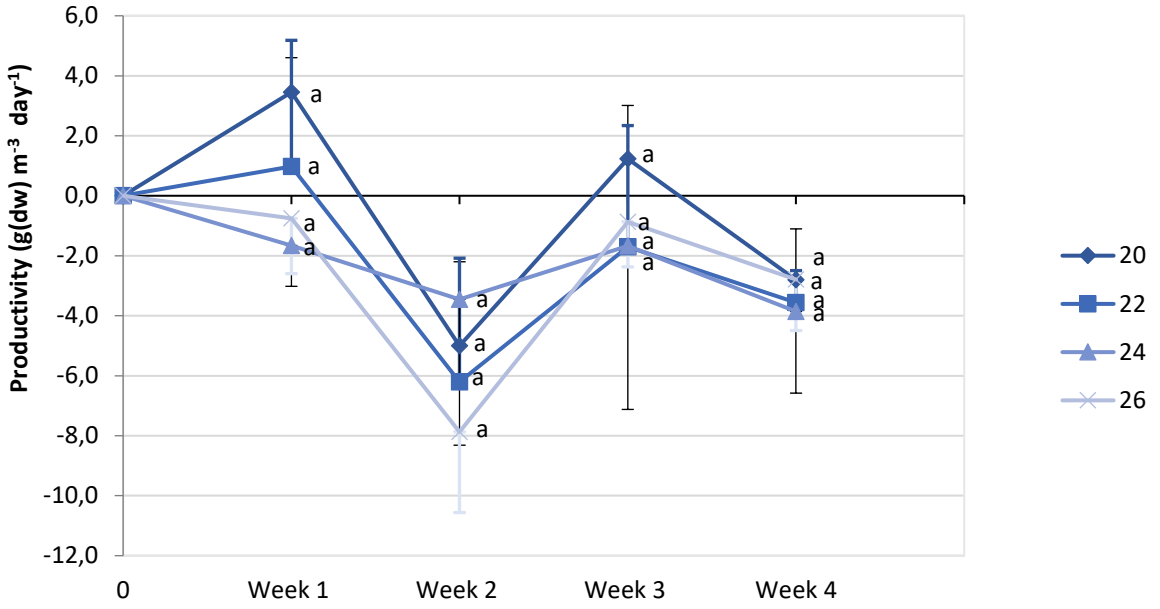


Figure 12- Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout four weeks, with four different temperatures, 20ºC, 22ºC, 24ºC, 26ºC. Different letters above near values, indicate statistical differences for the productivity between treatments in each week (ANOVA, *p*-value < 0.05).

3.1.6 Trial with wastewater from a meagre aquaculture

MW 100% showed better RGR (Figure 13) for Week 1 (1.21 ± 1.50% fw day^{-1}) and Week 3 (3.73 ±0.67 fw day^{-1}), while MW 50% presented higher percentages for Week 2 (7.38 ± 1.01% fw day^{-1}) and Week 4 (2.15 ± 0.72% fw day^{-1}). Nutribloom® showed lowest RGR for Week 1 (-0.19 ± 2.34% fw day^{-1}) and Week 4 (1.56 ± 0.91% fw day^{-1}). In terms of statistical analysis all of the assumption were met, and there were not found any statistically significant differences for Week 1 ($F_{(2)}$= 0.296; *p*-value= 0.754), Week 2 ($F_{(2)}$= 2.088; *p*-value= 0.205), Week 3 ($F_{(2)}$=3.219; *p*-value= 0.112), nor for Week 4 ($F_{(2)}$=0.177; *p*-value= 0.842).

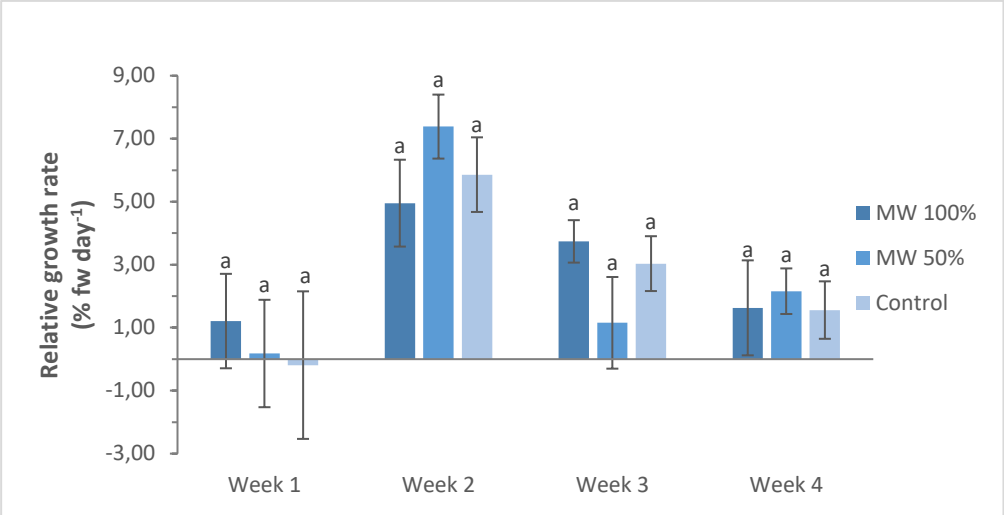


Figure 13- Relative Growth Rate (RGR) (Mean ± SD) on *Halopteris scoparia*, throughout four, with three different treatments, using wastewater from a meagre rearing, MW 100%, MW 50%, and a control. Letters above bars indicate statistical differences for the Relative Growth Rate between treatments in each week (ANOVA, *p*-value < 0.05)

For productivity (Figure 14), Week 1 showed that all groups started with relatively low productivity values, with MW 100% (3.69 ± 4.41 g dw $m^{-3}day^{-1}$) presenting the highest value and control the lowest (-0.01± 6.12 g dw $m^{-3}day^{-1}$). In Week 2 all the groups experienced a significant increase in productivity values, MW 100% (17.43± 3.91 g dw $m^{-3}day^{-1}$), MW 50% (27.00± 4.70 g dw $m^{-3}day^{-1}$) and Control (19.53± 3.51 g dw $m^{-3}day^{-1}$). For Week 3, it was observed a large decrease in MW 50% to a value of 5.23± 6.41 g dw $m^{-3}day^{-1}$. In Week 4, MW 100% (10.09± 8.97 g dw $m^{-3}day^{-1}$) and Control (7.86± 3.86 g dw $m^{-3}day^{-1}$) presented a decrease, while MW 50% (11.76 ± 3.91 g dw $m^{-3}day^{-1}$) showed an increase. There were no statistical differences found for Week 1 ($F_{(2)}$=0.282; *p*-value=0.764), Week 2 ($F_{(2)}$=3.048; *p*-value =0.122), Week 3 ($F_{(2)}$=3.541; *p*-value = 0.097) and Week 4 ($F_{(2)}$=0.207; *p*-value = 0.818).

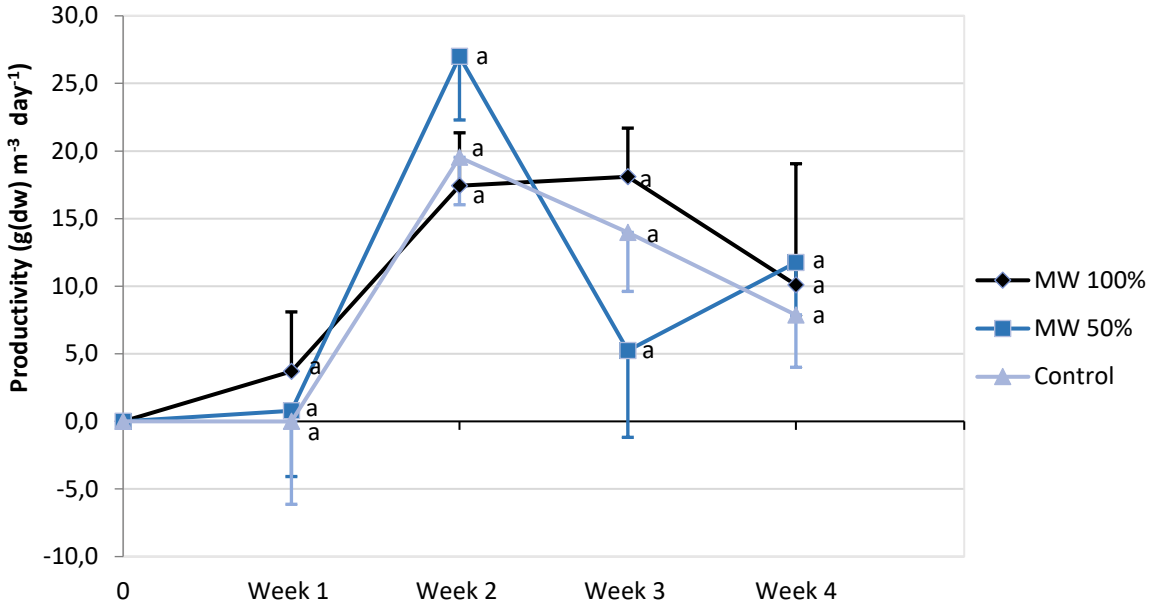


Figure 14- Productivity growth (g dw $m^{-3}day^{-1}$) of *Halopteris scoparia*, throughout four weeks, with three different treatments, using wastewater from a meagre rearing, MW 100%, MW 50%, and a control. Different letters near mean values indicate statistical differences for the productivity between treatments in each week (ANOVA, *p*-value < 0.05).

3.2 Ash and Moisture

The ash content was analysed for the wild algae, Salinity Trial, Density trial, Temperature trial and Meagre Wastewater Trial. Overall, wild algae presented higher value with 52.38± 6.51 %, for the salinity trial, presented a decrease in ash content with the decrease in salinity presenting with 37.76± 2.93 % at 35 psu, and 35.77± 2.09% at 25 psu. In the density trial, we can observe an increase in value with the increase of density peaking at 3g/L with a density of 44.61± 3.42 %.For the temperature trial, the ash content varied between 47.40± 2.72 % (20ºC) and 50.37± 0.88% (24ºC). Lastly the trial with seawater from a meagre aquaculture MW 100% and MW 50% presented similar values (36.59± 2.43% and 36.35± 5.83%, respectively).

For the statistical analysis, between the different categories (Wild Algae, Salinity 35 psu, Salinity 30 psu, Salinity 25 psu, Stocking density 1g/L, Stocking density 2g/L,

Stocking density 3g/L, MW 100% and MW 50%), there were found statistically significant differences ($F_{(12)}$=32.159; *p*-value= 0.024).

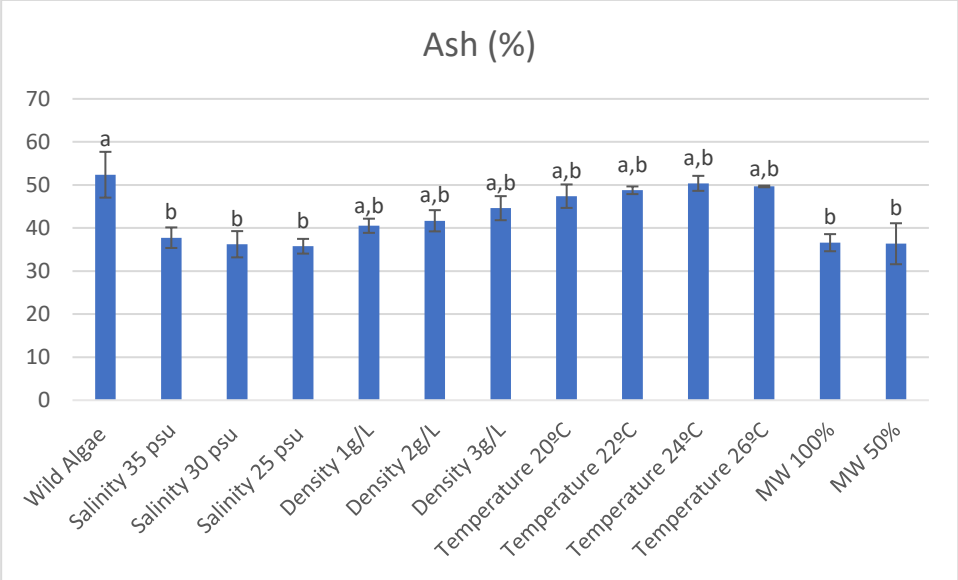


Figure 15 - Ash content of *Halopteris scoparia* expressed in percentage (Mean ± SD) for the Wild Algae, Salinity (35, 30, 25 psu), Density (1, 2 and 3 g/L), Temperature (20, 22, 24 and 26 ºC) and MWT (MW 100% and MW 50%). Different letters above the bars indicate statistical differences for the ash content between treatments for the different trials (Kruskal-Wallis, *p*-value < 0.05).

In terms of moisture content, the wild algae presented the lowest value when compared to the remaining trials (68.79 ± 1.18%). For the remaining trials, moisture content registered, within the trials, showed very similar values. The salinity trial ranged from 71.23± 0.85 % (25 psu) to 72.05± 0.23 % (35 psu), the density trial ranged from 71.70± 0.15 % (2g/L) to 71.74±0.62 % (3g/L), the temperature trial ranged from 71.49± 0.26% (20ºC) to 72.12± 0.30% (26ºC), and lastly the trial with seawater from a meagre aquaculture ranged from 72.49± 0.27 % (MW 100%) to 73.40± 0.52%(Control).

For the statistical analysis, between the different categories (Wild Algae, Salinity 35 psu, Salinity 30 psu, Salinity 25 psu, Stocking density 1g/L, Stocking density 2g/L,

Stocking density 3g/L, MW 100% and MW 50%), there were found statistically significant differences ($F_{(8)}$= 9.415; *p*-value< 0.001).

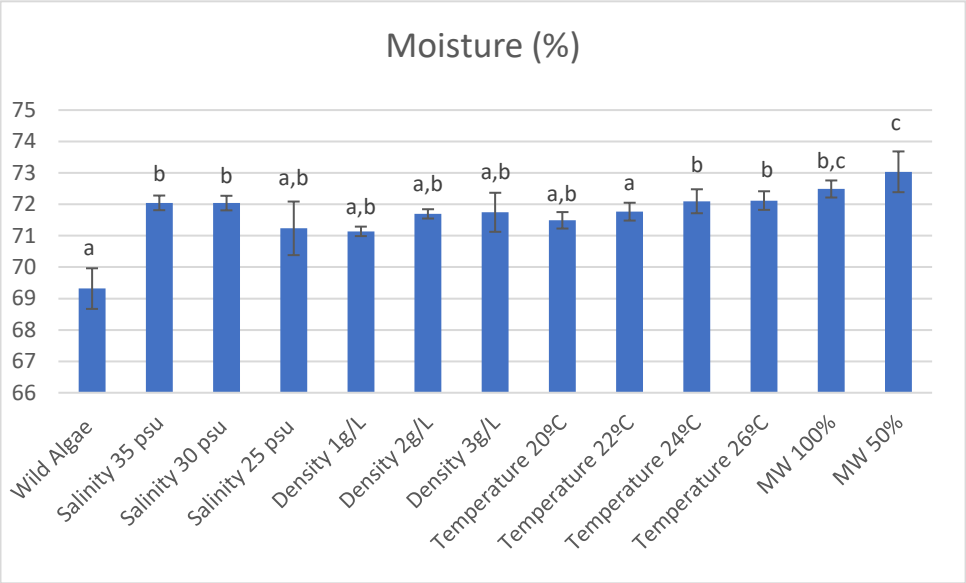


Figure 16 - Moisture content of *Halopteris scoparia* expressed in percentage (Mean ± SD) for the Wild Algae, Salinity (35, 30, 25 psu), Density (1, 2 and 3 g/L), Temperature (20, 22, 24 and 26 ºC) and MWT (MW 100% and MW 50%). Different letters above the bars indicate statistical differences for the Relative Growth Rate between treatments for the different trials (Kruskal-Wallis, *p*-value < 0.05).

4 Discussion

Halopteris scoparia was chosen for this study due its potential in various fields, including cosmetics, bioremediation, agriculture, and energy production. Despite its promising uses, the cultivation of *H. scoparia* has been explored to a limited extent, primarily by Patarra et al. (2017).Therefore, present study shows the growth dynamics of *H. scoparia* in different conditions, including different nutrient media, salinities, densities, temperature, and light wavelengths. Additionally, the response of the macroalgae to fish wastewater was also analysed, providing insights into its potential uses in integrated aquaculture systems. Lastly, the ash and moisture content were also analysed.

The data obtained allowed a deeper understanding of how different abiotic factors can influence the growth of *H. scoparia* and whether this macroalgae would be suitable for aquaculture production.

4.1 Nutrient trial

The trial results indicate significant differences in the Relative Growth Rates (RGR) of *Halopteris scoparia* between Weeks 1 and 2, with Nutribloom® consistently demonstrating more promising outcomes compared to the conventional Von Stosch Enriched nutrient medium (VSE). Productivity also shown the same tendency.

Nutribloom®, which has traditionally been used for microalgae, showed a higher RGR ranging from 2.06 ± 2.83% fw day^{-1} to 10.49 ± 1.07% fw day^{-1}, compared to VSE's results of 1.60 ± 0.55% fw day^{-1} to 4.71 ± 1.77% fw day^{-1}. These findings suggest that Nutribloom® has a more suitable composition for *H. scoparia* growth, highlighting the importance of the media composition for the growth of this macroalgae. Our study also revealed higher results than Patarra et al., (2017), having higher than 10% day^{-1} for growth rate and over 50g (dw) $m^{-3}day^{-1}$ for productivity.

For large-scale production, the use of a ready-to-use formula like Nutribloom® could offer substantial advantages by reducing labour costs and optimizing operational efficiency. This study highlights the potential of Nutribloom® for macroalgae cultivation, paving the way for further research on its broader applications in aquaculture systems.

4.2 Salinity trial

Despite the absence of statistically significant differences between the different salinity levels for the RGR for Week 1 and Week 2, it was still possible to observe some trends in the final results. For Week 1, a higher RGR percentage was observed for 25 psu. While Week 2, recorded a higher RGR percentage for 30 psu. *H. scoparia* presented the lowest RGR for the salinity level 35 psu, for Week 1 and Week 2.

The lack of statistical differences between the studied salinities may indicate that *Halopteris scoparia* can adapt to changes in the environment and can tolerate a wider range of salinities. This was also verified by Silva (2009) which concluded that *H. scoparia,* tolerates a wide range of salinities of 20 psu to 46 psu. Similarly, Connan & Stengel (2011), reported that other brown algae species, *Undaria pinnatifida* and *Fucus vesiculosus*, which showed resilience across a salinity range of 15 to 35 psu. This aligns with the characteristics of an intertidal macroalgae that are often subjected to harsh and variable environmental conditions.

According to Balogh & Byrne (2021), intertidal species are exposed to a wide range of salinities due to heavy rain, river discharge, and evaporation caused by hot air and strong winds increasing the salinity. These conditions may decrease or increase seawater salinity. Shore-based cultivation of macroalgae is similarly affected by these characteristics, which makes it essential for the cultivated species to have a higher adaptability to different salinities. The resilience of *H. scoparia* to varying salinity levels is advantageous when pairing it with other species in aquaculture systems, providing a wider range of choices for species that thrive under different salinity conditions.

4.3 Density trial

Densities of 1g/L and 2g/L showed significantly higher RGR when compared to 3g/L, in Week 1, indicates that *H. scoparia* has a better performance when in lower densities. Higher densities impact the photosynthesis and growth of macroalgae (Bao et al., 2024), leading to a higher competition for resources, light and nutrients (Praeger et al., 2020; Xiao et al., 2019), which can be a limiting factor for the growth of macroalgae including *H. scoparia*. This suggests that overcrowding can have negative effects for this macroalgae production.

This finding is consistent with Patarra et al., (2017), who also observed higher growth rates with lower densities, although they did not report statistical differences. Patarra et al., (2017) noted the highest RGR value for 0.075g fw L^{-1}, with a lower productivity, while 3g fw L^{-1} presented higher productivity. Overall, both studies highlighted that even though lower densities register higher RGR, an optimal productivity can be achieved at 2g/L.

The preference for lower densities is also highlighted in other species, like *Ulva linza*, in a study conducted by Bao et al. (2024) that experimented the densities of 0.2g/L, 1g/L and 2g/L. This study concluded that, even with high light conditions of 300 µmol.m-$^{2.}$ s^{-1}, higher densities significantly inhibit the growth of the species. Similarly, Abreu et al. (2011) studied *Gracilaria vermiculophylla*, with 3, 5 and 7 Kg m^{-2}, in IMTA systems, concluding that lower densities were beneficial for the macroalga growth.

This shows the importance of optimizing the stocking densities in macroalgae cultivation. While low densities can result in a higher growth rate, they may also lead to waste of resource, reduced yields and increasing production cost. Conversely, to high of a density will diminish the growth and even causing mortality. Thus, finding the right balance in stocking density is crucial for maximizing both growth rates and productivity in macroalgae farming.

4.4 Light trial

The results revealed distinct responses to the different LED lights colours in the growth of *H. scoparia*. Notably, White LED presented as the most favourable light source, as indicated by higher RGR when compared to Red and Blue LEDs lights. Statistically, there were differences between White LED light to both Red and Blue LEDs. Even though there were no significant differences between Red and Blue, the results revealed a tendency for a higher RGR, when *H. scoparia* was subjected to the Blue LED, when compared to the Red LED light.

Brown macroalgae, are able to manage excess light energy and oxidative stress due to photoprotective pigments, namely β-carotene and xanthophylls (Endo et al., 2023). In previous studies, *H. scoparia* was able to grow in light intensities up to 150 µmol photons m^{-2} s^{-1}. For the present study, a light intensity used of 60 µmol photons m^{-2} s^{-1} was used.

Several studies have been conducted on the influence of LED lights on brown algae. Miki et al. (2017) studied the influence of red and Blue LED lights on *Sargassum horneri* in germling and immature stages. This study revealed a higher growth rate when the macroalgae was under the Blue LED light. These results contradict a study conducted by Öztaşkent & Ak (2021) on brown algae, that revealed a higher growth rate in *Treptacantha barbata* when under the influence of the Red LED light. The study was conducted between Yellow, Blue, Green and Red LED lights and white fluorescent light as a control.

The tendency toward higher growth rates under Blue LED light observed in our study could be explained by the effects of Blue light (400-500nm) on enhancing cell proliferation and drive photosynthesis, making it a good addiction to promote plant growth. In addition, pure red light has been claimed to cause cell damage (Palanisamy et al., 2022). These could explain the tendency towards the better results obtained in the Blue LED light on our study.

For this study it was used LED lights, which show a narrower emission spectrum (20- 30 nm at half peak height) (Öztaşkent & Ak, 2021), and present a higher photon output which can promote a better growth of the algae (Glemser et al., 2016). On the other hand, fluorescent lights present a wide wavelength band, (350nm-750nm), which causes an energy waste and excessive temperatures, promoting photostress or damage to photosynthetic organisms (Gong et al., 2020).

4.5 Temperature trial

For the temperature trial, the RGR, varied significantly across the different temperature. Lower temperatures seemed to promote better growth, having only the lower temperatures present positive RGR (20ºC and 22ºC). Conversely, higher temperatures presented the lowest RGR (26ºC), suggesting that higher temperatures are less favourable for *H. scoparia* growth. These results could be influenced by the lower light intensity used for this trial, resulting in mostly negative RGR.

Previous studies have reported similar results regarding the effects of temperature on *H. scoparia*. Silva, (2009) reported similar results with higher growth in lower temperatures. According to Patarra et al., (2017), *H. scoparia* can grow within a temperature range of 14 to 24 °C. This range is significant as it indicates the species' adaptability to varying thermal conditions, which is essential for its cultivation and management in aquaculture settings. With 26ºC having an overall higher decreased in RGR, reinforces the idea that temperatures above 24ºC are suboptimal for *H. scoparia.*

It is important to further study the effect of temperature because it plays a crucial role in controlling the rate of photosynthesis in all photosynthetic organisms. Intertidal macroalgae such as *H. scoparia* are frequently exposed to elevated water and air temperatures. These conditions affect growth, photosynthesis, and reproduction of these macroalgae (Clark et al., 2013). Understanding the optimal range of temperatures for *H. scoparia* is important for optimizing growth conditions in aquaculture systems.

4.6 Trial with seawater from a meagre aquaculture

Lastly, it was analysed *H. scoparia* growth with residual water from a meagre aquaculture, with three different wastewater concentrations, MW 100%, MW 50% and the control group. Week 1 and Week 4 reported lower RGR and productivity values for the variant Control, while Week 2 and 3 presented lower Values for MW 100% and MW 50%, respectively. Higher productivity values were observed for Week 1 and Week 3 for MW 100% and for Week 2 and 4 for MW 50%.

When comparing it to the nitrate levels between weeks, they do not seem to be the cause of the growth and productivity fluctuations. In fact, when nitrates are higher, Week 1 (77.3 mg/L) and Week 3 (34.5 mg/L), growth and productivity are lower in MW 50%, for example, while when the nitrate levels are lower, Week 2 and 4 (41.0 mg/L and 35 mg/L, respectively) the growth and productivity are higher in MW 50%. This suggests that the nitrate levels in the experiment might not cause the growth and productivity fluctuations. Although, the fluctuations observed, there was never a decrease in growth, meaning that *H. scoparia*, presented a high adaptability to the change in nitrate concentration, which could be a positive note for the integration of this macroalgae in IMTA systems.

In previous studies we have observed that brown macroalgae have also benefited from its pairing with fish wastewater. Fossberg et al., (2018) has shown that Kelp presented a yield of 100% for fresh weight and of 60% for dry weight when compared to monocultured cultivations, highlighting the significant growth enhancement of macroalgae in nutrient rich waters. Macroalgae benefit from dissolved inorganic nitrogen present in fish wastewater, using it for macroalgae growth. This creates a symbiotic relationship where nutrients would otherwise contribute for water quality degradation (Hadley et al., 2016).

4.7 Ash and moisture content

Ash and moisture content was analysed for the wild macroalgae and cultivated macroalgae from the Salinity trial (35, 30 and 25 psu), Density trial (1, 2 and 3g/L), Temperature trial (20, 22, 24 and 26ºC) and Trial with seawater from a meagre aquaculture (MW 100% and MW 50%).

The ash content presented a decrease when cultivated exhibiting a percentage of 52.37± 6.51 % on wild algae while the cultivated biomass varied from 36.23± 3.73 % (Salinity 35 psu) to 50.38± 2.14% (Temperature trial 24ºC). In previous studies, it was observed that macroalgae ash content tends to increase when in cultivation conditions (Cardoso et al., 2023). Ash content can be influenced by multiple factors such as diatoms contaminations, growth media (Liu, 2017), mineral availability which, in turn, depends on several factors such as water salinity, sunlight, season and growth conditions.

When compared to wild algae the moisture content in *H. scoparia* presented an increase in percentage (69.32± 0.64%), reaching the highest value for the MW 50% (73.03± 0.64%), these results agree with Cardoso et al. (2023) who studied the moisture, ash and lipids contents of *Saccharina latissima*, also observed an increase in moisture after cultivation. Likely due to the tendency to retain more water when presented with optimal growth conditions.

5 Conclusion

Halopteris scoparia presents a significant potential for cultivation in aquaculture systems due to its adaptability and high growth rates. The introduction of Nutribloom® played a crucial role in achieving higher growth rates and biomass production results, outperforming previous studies. Optimizing stocking densities was found essential to maximize productivity, lower densities minimize overcrowding and competition for light and nutrients, resulting in better macroalgae growth. In terms of salinity, the macroalgae, showed adaptability to the different salinities, which can be beneficial for aquaculture systems, particularly those exposed to fluctuating coastal conditions. White LED lights obtained better results, compared to Red and Blue LED's, further contributing for its viability for *H. scoparia* cultivation. However, the effects of temperature remain inconclusive, being necessary further studies to observe its effects on *H. scoparia.*

Remarkably, our results showed promising possibilities for the cultivation of *H. scoparia* with fish wastewater, showing that this macroalgae is capable of growth, while recycling nutrients and improving the water quality for other possible species and reducing pollution caused by monocultured fish productions. Further testing needs to be conducted to determine the long-term viability of using fish wastewater for cultivating *Halopteris scoparia*, especially regarding its role in nutrient cycling, water quality improvement, and its interactions with other species in integrated aquaculture systems. Additionally, future studies should focus on assessing the scalability of these cultivation methods for commercial use, as well as examining the effects of seasonal changes, temperature, and light intensity on the growth and nutrient absorption of the macroalgae.

Cultivated *H. scoparia* exhibited higher ash content and lower moisture content when compared to the wild specimen, these differences seem to be driven by cultivation conditions. Further studies need to be conducted to better understand the algae biochemical composition.

6 Bibliography

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