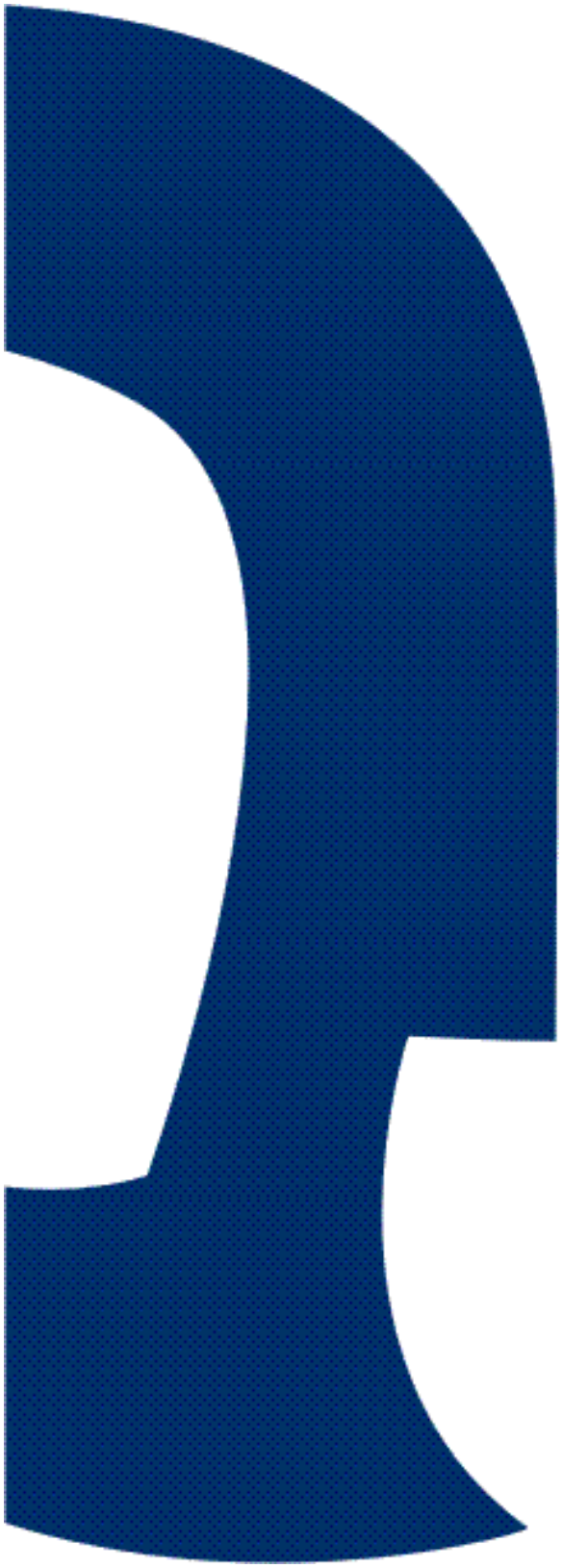

FACULTAD DE CIENCIAS DEL MAR

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Intensive cultivation of *Palmaria palmata* and evaluation of its chemical composition

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Abstract

Seaweed based Integrated Multi-Trophic Aquaculture (IMTA) systems are being designed to mitigate the environmental problems caused by several forms of fed aquaculture. A seaweed cultivation system using 127 and 230 L tanks was used to cultivate *Palmaria palmata* (Rhodophyta). *Palmaria palmata* is a cold temperate species of the North Atlantic and North Pacific Oceans. In the present study *Palmaria palmata* could grow throughout the entire course of the experiment and under all conditions tested. The highest RGR value (3,13 % ± 0,40) was observed from *Palmaria palmata* cultivated under a density of 1 kg m^{-2}. Under winter conditions the productivity value reach the maximal value of 26,89 ± 19,02 g(dw) m^{-2} $week^{-1}$. The total lipid, protein, ash, carbohydrates and individual fatty acid contents of *Palmaria palmata* were determined (fatty acids by gas chromatography). Total lipid content ranged from 0,00389 ± 0,0003 to 0,00987 ± 0,00030 (% ± se). The most abundant fatty acids were C16:0, C18:0 and C20:5. Ash content ranged from 14,5 ± 0,67 to 24,2 ± 0,86 (% ± se), protein content from 17,3 ± 0,05 to 24,8 ± 0,02 (% ± se) and carbohydrates from 55,52 to 67,56%.

Key words: *Palmaria palmata*, cultivation, productivity, chemical composition, fatty acids.

Resumen

La Acuicultura Multi-Trófica Integrada (AMTI) está basada en algas para mitigar los problemas ambientales causados por los procesos desarrollados en la acuicultura moderna. Se utilizó un sistema de cultivo de algas utilizando tanques de 127 y 230 L para cultivar *Palmaria palmata* (Rhodophyta). *Palmaria palmata* es una especie de temperatura fría del Atlántico Norte y del Océano Pacífico Norte. En este estudio, *Palmaria palmata* fue capaz de crecer a lo largo de todo el experimento y bajo todas las condiciones testadas. El mayor valor de la tasa relativa de crecimiento (3,13% ± 0,40) se observó a partir de *Palmaria palmata* cultivada bajo una densidad de 1 kg m^{-2}. En condiciones invernales, el valor de productividad alcanza el valor máximo de 26.89 ± 19,02 g (peso seco) m^{-2} $semana^{-1}$. Se determinaron los contenidos totales de lípidos, proteínas, cenizas, carbohidratos y ácidos grasos individuales. (Ácidos grasos mediante cromatografía de gases). El contenido total de lípidos osciló entre 0,00389 ± 0,0003 y 0,00987 ± 0,00030 (% ± se). Los ácidos grasos más abundantes fueron C16:0, C18:0 y C20:5. El contenido de cenizas varió de 14,5 ± 0,67 a 24,2 ± 0,86 (% ± se), contenido de proteína de 17,3 ± 0,05 a 24,8 ± 0,02 (% ± se) y carbohidratos de 55,52 a 67,56%.

Palabras clave: *Palmaria palmata*, cultivo, producción, composición química, ácidos grasos.

1.Introduction

The word algae is used to designate a large, varied, and heterogeneous group of organisms that, at present, do not have a clear-cut, formal taxonomic status. Some scientists have estimated that there might be between one and ten million different species, by far the majority of which have not yet been described. Algae come in many different sizes. The smallest of them, the microalgae, are unicellular and make up what we call plant (or phyto) plankton. The largest algae are multicellular organisms, growing to lengths of up to 60 meters, which can form enormous 'forests' in the ocean. These large marine algae, which are also referred to as macroalgae, are the ones that most people associate with the word seaweeds (Mouritsen, 2013). The systematic organization of algae into Phyla and Classes is based on several characteristics, such as the type of photosynthetic pigments, storage compounds, type of cell wall and cellular ultrastructure. Marine macroalgae belong to a polyphyletic group, divided into three main phyla: Ochrophyta (Class Phaeopyceae), Chlorophyta, and Rhodophyta, which are commonly referred to as the brown, the green, and the red algae (Gallardo, 2014).

The major environmental factors affecting seaweeds are light, temperature, salinity, water motion, and nutrient availability. Light quality and quantity, which are important in photosynthetic responses and metabolic patterns, both change with depth, but the changes depend mainly on turbidity. Salinity is another complex factor, of which the two chief components are the osmotic potential of the water and the ionic composition. Osmotic potential affects water flow in and out of the cell, turgor pressure, and growth, while the concentrations of Ca^{2+} and HCO_3^- affect membrane integrity and photosynthesis, respectively. The hydrodynamic aspects of water motion are critical to thallus survival on wave-swept shores and to spore settling, and water motion also has important effects on the boundary layers over plant surfaces and thus on nutrient uptake and gas exchange. Nutrients must be considered not simply in their absolute concentrations but also in the amounts present in biologically available forms; concentrations of trace metals may create toxicity problems, particularly in polluted areas. Temperature and salinity affect the density of seawater, hence the mixing of nutrient-rich bottom water with nutrient-depleted surface water and water motion can affect turbidity and siltation as well as nutrient availability (Lobban and Harrison, 1994).

According to FAO (2016) the production of aquatic plants from aquaculture in 2014 amounted to 27.3 million tonnes of (US$5.6 billion). Aquatic plant farming, overwhelmingly of seaweeds, has been growing rapidly and is now practised in about 50 countries. Seaweeds are industrially processed to extract thickening agents such as alginate, agar and carrageenan or used, generally in dried powder form, as an animal-feed additive. Growing attention is also focusing on the nutritional value of several seaweed species, due to their abundance of natural vitamins, minerals, and plant-based protein. Many seaweed flavoured foods (including ice creams) and drinks are being launched,

with the Asia and Pacific region as main market, but with increasing interest also being shown in Europe and America. However, seaweeds are characterized by a highly variable composition, depending on species, collection time and habitat. More research is also exploring the use of seaweed as an alternative to salt. Procedures are being developed for the industrial preparation of biofuel from fish waste and seaweeds.
The methods of seaweed cultivation are greatly varied. The two main types of large-scale seaweed cultivation are in open-sea or land-based systems (Abreu and Champenois, 2015).

Open water seaweed cultivation does not require land area, and allows larger biomass production compared to land based systems. On the other hand, advantages of land-based cultivation include better control of the cultivation system, easy access to the produced biomass regardless the weather conditions, and potential to be used for bioremediation of land-based fed aquaculture (IMTA). In such systems, it is possible to manipulate key factors such as light intensity and nutrient loading and thus, have a higher control over biomass yield, chemical composition and epiphytes (Harrison and Hurd, 2001).

To date, the most promising form of aquaculture is integrated multi-trophic aquaculture (IMTA), a system in which several species from different levels in a food chain or a food web are placed in close proximity to each other. The idea is to mimic naturally occurring ecosystems to create an optimal balance between the various elements in it. An example of such a system could consist of an organism that is raised on fodder and, in turn, gives off both organic and inorganic waste products that provide nutrients for, or are extracted by, complementary species. Experimental IMTA projects combining farmed fish, shellfish, and seaweeds have shown that such systems can increase the total output by up to 50% over that obtained from the monoculture of each. In addition, co-cultures have the potential to mitigate the conditions that lead to eutrophication and harmful algal blooms. This is an exciting prospect, given that fish farms are currently the fastest growing sector of global food production, with the yield increasing fewer than 10% annually. Comparable figures for the catch of wild fish and the production of meat are 1.4% and 2.8%, respectively (Mouritsen, 2013).

In the present study, the chemical composition and density controlled test of *Palmaria palmata* cultivated in IMTA system were carried out to determine which are the best conditions for fast production and the best nutritional values. *Palmaria palmata* (Linnaeus) O. Kuntze (1981) is a red seaweed (Rhodophyta, Order Palmariales) and is commercially known in the food sector as Dulse. It has a flat thallus which is typically divided in a fork-like manner (dichotomously) or grows palmately, i.e. into lobes radiating from the centre of the fronds. Mature fronds commonly grow to about 50 cm but can reach a length of up to one metre and the texture of fronds is membranous or

leathery (Werner and Dring, 2011). *Palmaria palmata* is a cold temperate species of the North Atlantic and North Pacific Oceans. The area of distribution in the North Atlantic stretches from Spitzbergen and Greenland in the Arctic (80°N) to Portugal and New Jersey (40°N). *Palmaria palmata* is also found along the Pacific coasts of Canada and the USA from Alaska to northern California, and along the cold-temperate coasts of eastern Russia (Sakhalin and the Kuril Islands) and northern Japan (Lüning, 1990). It is a common constituent of many rocky shores in Ireland and the UK (Bunker et al., 2010; Hardy and Guiry, 2003). It grows in the lower intertidal and shallow subtidal zone to a maximum depth of 20 metres. In European waters, the algae is a frequent epiphyte on stipes of *Laminaria hyperborea* or *Laminaria digitata*, but it is also found occasionally on Fucus species or on rocks and mussel shells. *Palmaria palmata* prefers sheltered to semi-exposed sites with a moderate to strong water current. A good water flow has a very positive effect on growth, probably because of its impact on nutrient exchange. Young fronds of Palmaria are normally free of epiphytic growth, but older fronds are frequently covered by bryozoans and other organisms. Older thalli also frequently contain small endophytic algae, which are visible as dark brown dots in the tissue, and marine fungi (Werner and Dring, 2011).

Algae are promising organisms for providing both novel biologically active substances and essential compounds for human nutrition (Tringali, 1997; Burja et al., 2001; Mayer and Hamann, 2004). Therefore, an increasing supply for algal extracts, fractions or pure compounds for the economical sector is needed (Dos Santos et al., 2005). Fatty acids with two or more methylene-interrupted double bonds are essential for normal cell function, and have entered the biomedical and nutraceutical areas as a result of elucidation of their biological role in certain clinical conditions common in Western society such as obesity and cardiovascular diseases (Gill and Valivety, 1997; Sayanova and Napier, 2004). Moreover, polyunsaturated fatty acids (PUFAs) play key roles in cellular and tissue metabolism, including the regulation of membrane fluidity, electron and oxygen transport, as well as thermal adaptation (Funk, 2001). In addition, public perception of healthy food and life style has brought them to the attention of the consumer. In particular, there is increasing interest in a typical PUFA family (ω-3) named eicosapentaenoic acid (EPA, C20:5$\Delta^{5,8,11,14,17}$,20:5 ω-3) (Cardozo et al., 2007). The n-6: n-3 ratio, which is currently recommended by the WHO (World Health Organisation) to be lower than 10 in the diet, can possibly be improved by addition of certain edible seaweeds because of their high n-3 content (Van Ginneken et al., 2011). *Palmaria palmata* is considered as one of the more delectable seaweed for human consumption with a large and fairly unexplored potential for use in the cuisine (Mouritsen, 2012), both in the home kitchen (Rhatigan, 2009) and in gastronomy (Mouritsen et al., 2012). Dulse harvested in the wild, dried, and packed in closed plastic bags is sold commercially by a number of small companies, for

example, in Ireland, Brittany, Spain, Iceland, Maine, Nova Scotia, and California. There is a small production of cultivated dulse in Ireland, Brittany, Spain, Hawaii, and most recently Denmark (Mouritsen et al., 2013). In Europe, the most successful attempt to cultivate dulse takes place in the open sea along the coastline of northern Spain, an endeavor that is now responsible for the largest commercial production of red algae outside of Asia (Martínez et al. 2006).

Also, *Palmaria palmata* is reported to contain 11.9–21.9% protein per dry biomass, depending on seasonality and collection site, and it contains almost all essential amino acids as well as several mycosporine-like amino acids. It also contains carotenoids, including a-carotene, b-carotene and lutein, sterols such as cholesterol and desmoste- rol, D-homocysteic acid, kainic acid and 10-hydroxykainic acid (Banskota et al., 2014).

The main objectives of the present study were focused on (1) the evaluation of optimal growth parameters for the macroalgae *Palmaria palmata* under tank intensive culture conditions and (2) to analyse chemical composition of biomass obtained under IMTA-nutrient enriched compared to wild harvested algae.

2.Materials and methods

2.1 Algal material

Palmaria palmata was collected from the coast in Portugal in 2015 and stored in the culture chamber by ALGAplus. In October 2016, it was produced in their aquaculture system at Ria de Aveiro (Portugal, 40°36′43″N, 8°40′43″W).

2.2. The cultivation system

Palmaria palmata was cultivated in an integrated multi-trophic aquaculture (IMTA) system. Red and black polyethylene tanks of 127 L and 230 L (0,35 m^2 and 0,56 m^2) respectively were set to receive independent flows of water (seawater filtrated from the fish tanks) and used to grow the seaweed. The water flow was adjusted manually every day for each tank with 6 renovations per day. The seaweed was in constant movement by air diffusers placed in the middle of the bottom of the tanks. This helped to maximize the seaweeds exposition to light and nutrients in the water. The cleaner effluent from the seaweed tanks was re-introduced into the fish water system (Abreu, et al., 2011)

2.3. Environmental parameters

Water temperature and salinity were monitored during the cultivation phase. Everyday these parameters were measured with a multi- parametric sensor (multi 340i/set, WTW,Germay). The incident sunlight was also monitored and irradiance was registered

with a spherical light sensor (Spherical Quantum Scalar Sensor Mod. QSL-2100 Biospherical Instruments-Inc, USA) as described in (Abreu et al., 2011)

2.4. Experimental design

The algae were transferred from the laboratory to tanks of 127 L and 230 L depending of the quantity of the biomass. From the first week to the 6th week the algae was in an acclimatization time in the 7th and 8th week, the stocking density was controlled of 1kg/ m^{-2} with 6 water renovations, and in the 9th week the stocking density was changed to 2 kg/ m^{-2} also with 6 water renovations (Fig. 1).

2.5 Calculations of growth and production parameters

Every week, the biomass was totally removed from the tanks and distributed in plastic baskets. To drain the excess of superficial water, biomass was centrifuged and weighted, obtaining the fresh weight. Before restocking the tanks, we adjust the density for the experiment. The relative growth rate (RGR) of the seaweeds in the tanks was determined by the formula:

$\mu = 100 \ln (FW / IW) / t$

Where μ = RGR (% day^{-1}), FW= final weight in t days, IW= initial weight and T= days in culture

The productivity of the tanks was calculated by the equation:

$$P = ([(Nt - N0) / t * (DW/FW)] / A$$

Where P = production rate (g of dry weight m^{-2} day^{-1}), Nt-N0= excess of algae at day t, DW/FW= dry weight / fresh weight and A = Area of the tanks as described in (Abreu et al., 2011)

2.6 Chemical analysis

2.6.1 Biomass

Dried samples (25 °C, up to 12% moisture content) of *Palmaria palmata* were provided by ALGAplus Ltd. (production site located at Ria de Aveiro, mainland Portugal, 40°36′43″N, 8°40′43″W). The biomass was collected in different environments: wild, culture chamber and IMTA tank.

2.6.2. Reagents

HPLC grade chloroform and methanol were purchased from Fisher Scientific Ltd. (Loughborough, UK). All other reagents were purchased from major commercial sources. Milli-Q water (Synergy, Millipore Corporation, Billerica, MA, US)

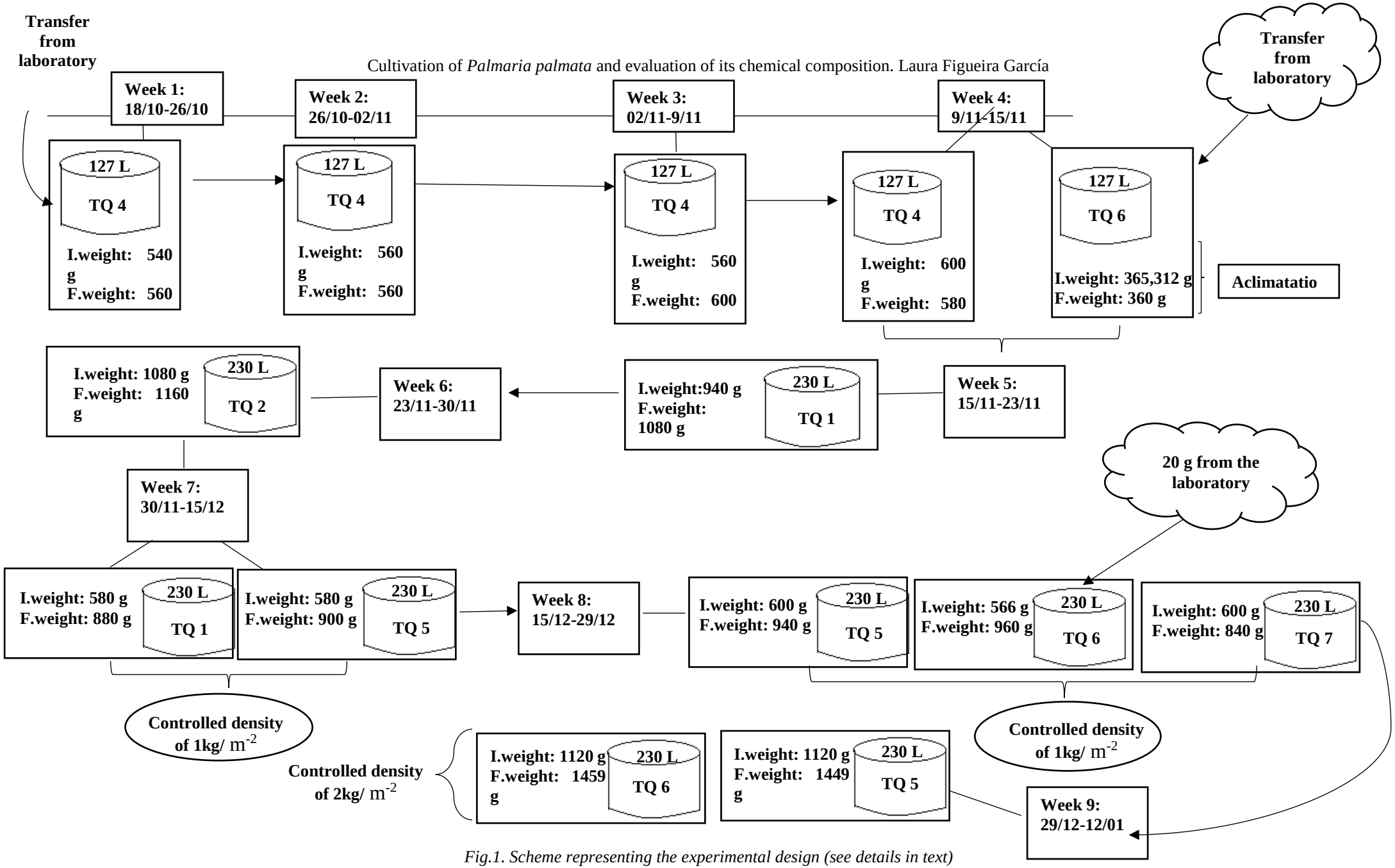

Fig.1. Scheme representing the experimental design (see details in text)

2.6.3. Lipid extraction procedure

According to protocols established at the Dpt. Chemistry (University of Aveiro) a mixture of chloroform/methanol (1:2, v/v) was added to 250 mg of dry weight seaweed. The mixture was transferred to a glass tube with a Teflon-lined screw cap and, after the addition of 3.75 mL of solvent mixture, it was homogenized by vortexing 2 min and then incubated in ice on an orbital shaker for 2 h and 30 min. The mixture was centrifuged at 2000 rpm for 15 min and the organic phase collected. The biomass residue was re-extracted twice with 1.5 mL of solvent mixture and 2.3 mL of water was added to the total collected organic phase to induce phase separation. Following this procedure, samples were centrifuged for 15 min at 2000 rpm, and the organic (lower) phase was collected to a new tube. 4 biological replicates were performed, with extractions and analyses taking place in different days. Lipid extracts were dried under a stream of nitrogen gas and lipid content was estimated as (%) of dry weight. Lipid extracts were stored at -20 °C prior to analysis.

2.6.4. Fatty acid analysis by gas chromatography-mass spectrometry (GC-MS)

Fatty acid methyl esters (FAMEs) were prepared from lipid extracts using a methanolic solution of potassium hydroxide (2.0 M). Volumes of 2.0 µL of the hexane solution containing FAMEs were analyzed by gas chromatography-mass spectrometry (GC-MS) on an Agilent Technologies 6890 N Network (Santa Clara, CA) equipped with a DB-FFAP column with 60 m of length, 0.25 mm of internal diameter, and 0.25 µm of film thickness (J&W Scientific, Folsom, CA,USA). The GC equipment was connected to an Agilent 5973 Network Mass Selective Detector operating with an electron impact mode at 70 eV and scanning the range m/z 40-500 in a 1 s cycle in a full scan mode acquisition. The oven temperature was programmed from an initial temperature of 80 °C, a linear increase to 155 °C at 15 °C/min, followed by linear increase at 8 °C/min to 210 °C, then at 30 °C/min to 250 °C, standing at 250 ºC during 18 min. The injector and detector temperatures were 220 and 280 °C, respectively. Helium was used as carrier gas at a flow rate of 0.5 mL/min. The identification of each FA was performed by mass spectrum comparison with those in Wiley 275 library and confirmed by its interpretation and comparison with the literature. The relative amounts of FAs were calculated by the percent area method with proper normalization considering the sum of all areas of the identified FAs (Da Costa et al., 2017).

2.6.5. Ash content determination

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 ^{o}C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and

N_2. The algae sample is weighed before and after ashing to determine the concentration of ash present. The ash content can be expressed on either a *dry* or *wet* basis:

$$\%\ \text{Ash (dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

$$\%\ \text{Ash (wet basis)} = \frac{M_{ASH}}{M_{WET}} \times 100$$

where M_{ASH} refers to the mass of the ashed sample, and M_{DRY} and M_{ASH} refer to the original masses of the dried and wet samples. (http://people.umass.edu/~mcclemen/581Ash&Minerals.html)

2.6.6. Protein determination:

The protein determination of *Palmaria palmata* samples was carried out in duplicate. Protein was measured by combustion method (Truspec 630-200-200) and measured as elemental nitrogen multiplied by a conversion factor calculated based on the ratio of proteinaceous and non-proteinaceous nitrogen in the biomass. Combustion furnace temperature of 1075ºC, afterburner temperature of 850 ºC. Nitrogen was determined by thermal conductivity. Two factor were used for converting total nitrogen to protein, 5 according to the literature (Angell et al., 2016) and 6.25 according to most of the papers like (Galland-Irmouli et al., 1999) and the traditional value used in seaweed trading and commercial activity.

2.6.7. Carbohydrates determination:

The carbohydrates were determined by subtracting 100 % from the sum of the protein, lipid and ash percent values.

3.Results:

3.1. Environmental parameters

3.1.1 Temperature

Data collected from a multi-parametric sensor (Fig. 2) show the daily variation of the temperature during the cultivation period, where the maximum value 19,25 °C was registered on 21th of October and the minimum value 7,1°C was registered on 20th of January

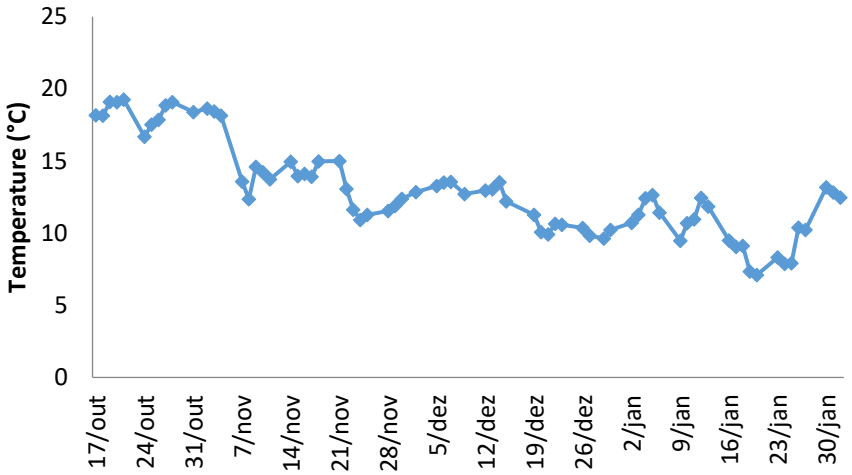

Fig. 2. Temperature data obtained with a multi- parametric sensor (multi 340i/set, WTW,Germay)

3.1.2 Salinity

With the multi-parametric sensor salinity was measured and the data obtained show the daily variation of the salinity during the cultivation period (Fig. 3), where the maximum value 38,1‰ was registered on 21th of October and the minimum value 31,42‰ was registered on 28th of November.

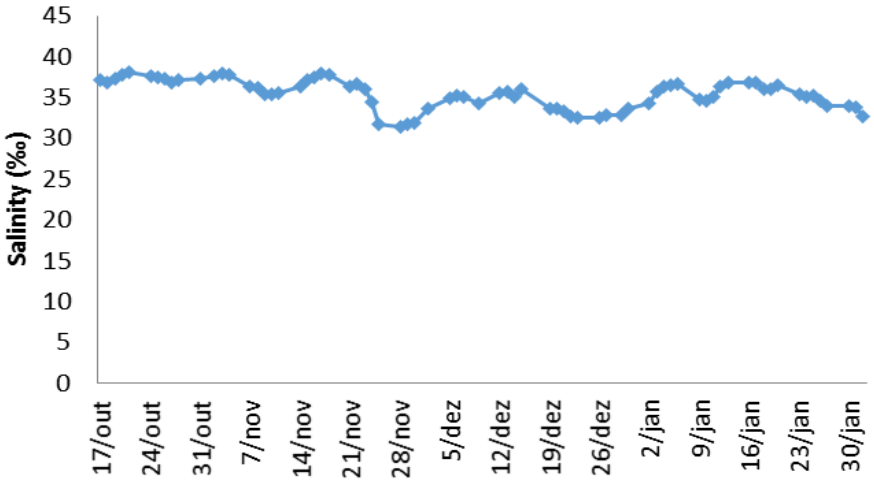

Fig. 3. Salinity data obtained with a multi- parametric sensor (multi 340i/set, WTW,Germay)

3.2. Growth performance

Palmaria palmata was able to grow in the IMTA system throughout the entire course of the experiment and under all conditions tested (Fig. 4-B) except for the periods of acclimation after transferring the algae from the cultivation chamber to the tanks outside when negative growth values were registered (Fig. 4-A).

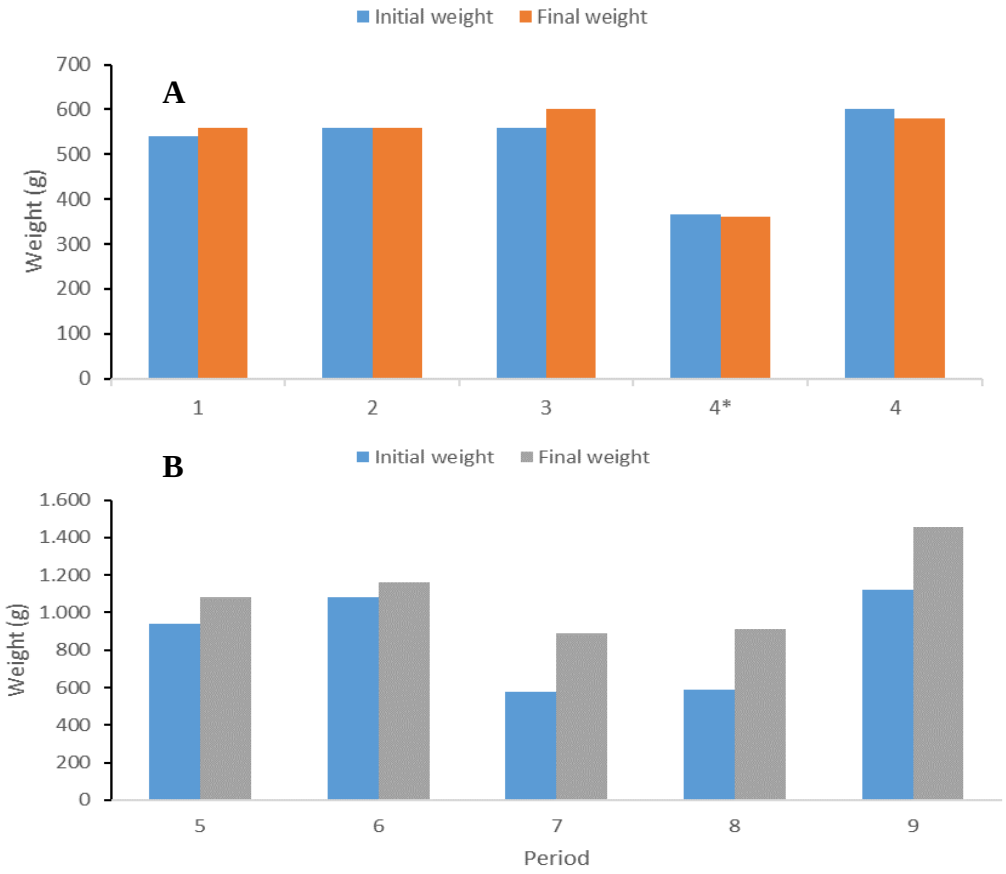

Fig. 4. A- Initial and final weight during the acclimation period. B- Initial and final weight during the experimental period

The highest RGR value (3,13 ± 0,40 % d^{-1}) was observed in period 8 (from 15/12/2016 to 29/12/2016) from *Palmaria palmata* cultivated under a density of 1 kg m^{-2}. The lowest value occurred in period 6 (from 23/11/2016 to 30/11/2016) in the tanks stocked with 1,93 kg m^{-2} (Fig. 5-A). Observing our weather registers it was a raining period with salinity values of 32,56 ± 1,16 ‰ (mean ± sd). In terms of yield, the results were slightly different. Although the lowest density showed high yield, there were some exceptions. Under winter conditions (during period 9 from 29/12/2016 to 12/01/2017), the yield value reach the maximal of 1270,39 ± 19,02 g (dw) m^{-2} $week^{-1}$. Also, the lowest value 434,69 g (dw) m^{-2} $week^{-1}$ was observed in period 6 (the same was observed when RGR data was analysed.) (Fig. 5-B)

3.3 Nutritional value

General composition of all the samples are shown in Fig. 6. As is show in the Fig. 6-A and B the sample of 21/10/2016 has the same composition in protein, ash and carbohydrates as the sample collected at 16/11/2016, both from the same period of the year. The only different is in the lipid content in which the sample for the culture chamber (Fig. 6 A) has less lipid content than the sample collected from the tank (Fig. 6-B). As opposed the samples collected the same day (16/11/2016) one from IMTA tank (Fig. 6-B) and the other from the culture chamber (Fig. 6-C) have different content of protein and carbohydrates but same content of lipids and ash.

On the other hand, the samples collected on the same period of the year (Fig. 6-D and E) are totally different, in which *Palmaria palmata* from the IMTA tank has more protein, lipids and ash than the wild.

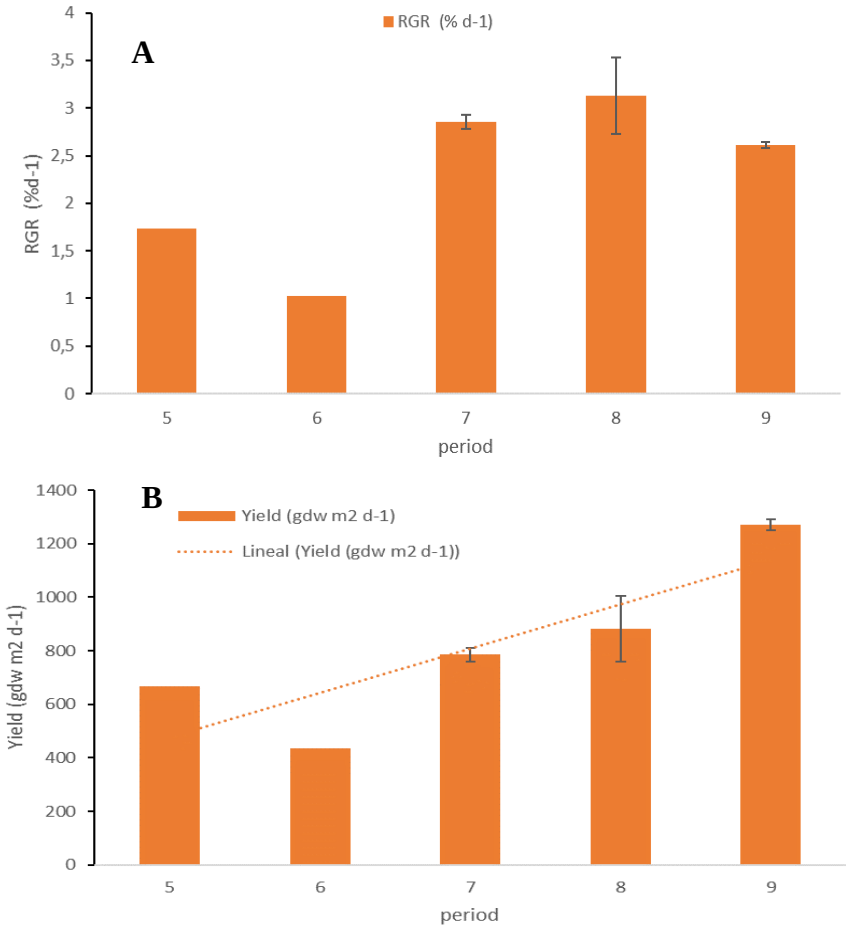

Fig. 5. A- Growth rate expressed in % d^{-1}during the experimental period. B- Yield (g dw m^{-2} d^{-1}) during the experimental period and in dots the tendency line.

3.3.1Protein content

Two conversion factors where used to measure the protein content: 5 according to (Angell et al., 2016) and 6,25 the most commonly used value (Table 1). Based on the latest, the protein amount of these samples varied from 17,3 ± 0,05 to 24,8 ± 0,02% of the dry mass (Fig. 7) where the sample from the culture chamber at the beginning of the experiment has the highest value and the sample wild the minimum.

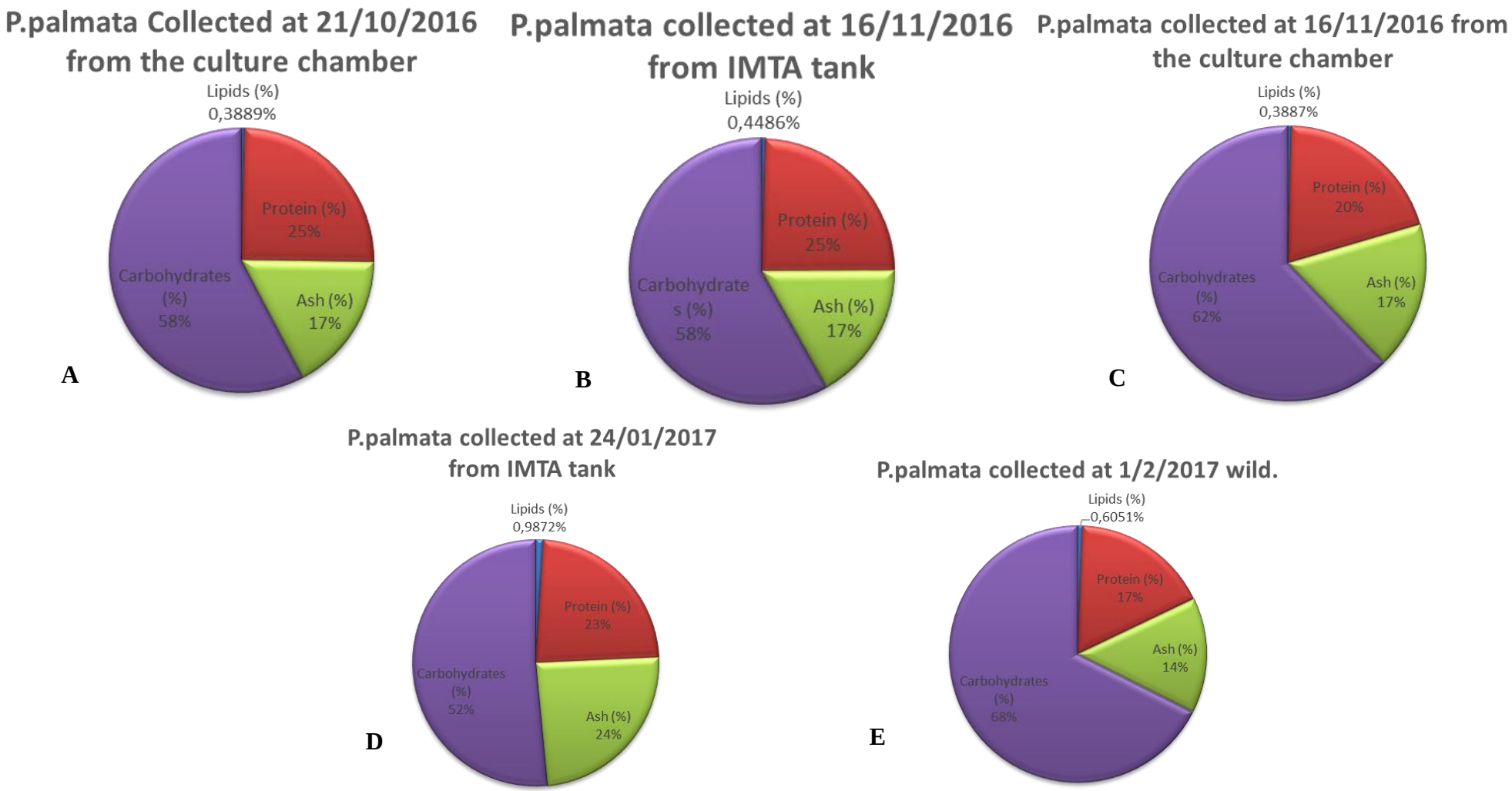

Fig. 6. Nutritional value of the different samples; A- for sample of 21/10/2016, B- for sample of 16/11/2016 (IMTA tank), C- for sample of 16/11/2016 (Culture chamber), D- for sample of 24/01/2017 and E- For sample of 1/2/2

*Table 1. Mean ± standard error (n=2). *(n=3) Protein content A) With the conversion factor used in paper: "the protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five" b) Conversion factor traditionally used in seaweed trading and commercial activity.*

	% N (mean)	**Protein (N x 5) (mean ± se) (a)**	**Protein (N x 6,25) (mean ± se) (b)**
P.palmata 21/10/2016 *(*culture chamber)	3,97	19,87 ± 0,02	24,8 ± 0,02
P.palmata 16/11/2016 (IMTA *tank*)	3,97	19,57 ± 0,01	24,5 ± 0,01
P.palmata 16/11/2016 (culture chamber)	3,21	16,05 ± 0,02	20,1 ± 0,02
P.palmata 24/01/2017 (IMTA *tank*)*	3,73	18,63 ± 0,29	23,3 ± 0,29
P.palmata 1/02/2017 (wild)	2,76	13,82 ± 0,05	17,3 ± 0,05

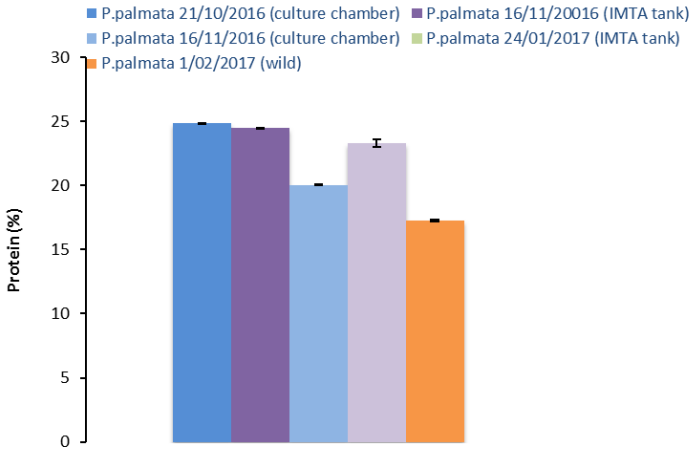

Fig. 7. Protein percentage of the samples analysed using 6,25 conversion factor. Vertical lines represent the standard error. (n=3).

3.3.2 Fatty acids

The most abundant fatty acids founded in *Palmaria palmata* were C16:0, C18:0 and C20:5 (Fig. 8).

Palmitic acid (C16:0) was measured in all samples at relatively high concentration. The lowest absolute value was measured in the sample collected at 24/01/2017 from IMTA tank (26,16 ± 0,67 % of total FA) and the highest absolute value in the sample collected at 21/10/20016 from the culture chamber (33,92 ± 0,29 of total FA). The important n-3 PUFA a-linolenic acid (C18:3) only appears in the sample from IMTA tank collected at 24/01/2017 (5,47 ± 0,06%) and another n-3 PUFA acid (C20:4) also appears in sample D (2,58 ± 0,02) and the sample collected from the wild at 01/02/2017 (2,77 ± 0,10). The important n-3 PUFA eicosapentaenoic acid (EPA, C20:5) was the most abundant FA in *Palmaria palmata*, being the maximum value in the sample C (collected at 16/11/2016 from the culture chamber) with a value of 45,50 ± 0,97 % of total FA, (Table 2). The n-6 FA arachidonic acid (C20:4) was found in the highest concentration in sample B collected at 16/11/2016 from the IMTA tank (4,14 ± 0,14 % of total FAs) the minimum value in

sample D (1,31 ± 0,09%). The n-6: n-3 ratio in seaweeds was ≤ 1.0 in sample D and >1 in the sample collected from the wild.

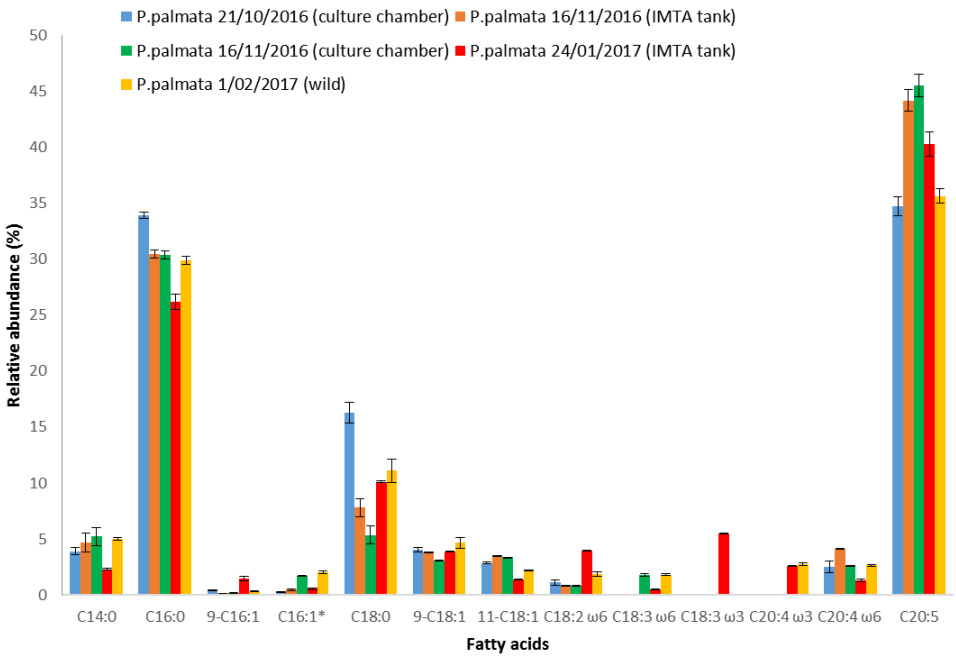

Fig. 8. Relative abundance of fatty acids in the samples analysed expressed in %. Vertical lines represent the standard error. (n=3)

Table 2. Mean ± Standard error (n=3). Relative abundance of fatty acids in the samples analysed expressed in % ± standard error

Fatty acid (%)	P.palmata (A)	P.Palmata (B)	P.palmata (C)	P.palmata (D)	P.palmata (W)
C14:0	3,90 ± 0,32	4,67 ± 0,40	5,21 ± 0,81	2,30 ± 0,0706	5,02 ± 0,12
C16:0	33,92 ± 0,29	30,41 ± 0,52	30,35 ± 0,35	26,16 ± 0,6756	29,90 ± 0,34
9-C16:1	0,39 ± 0,03	0,16 ± 0,02	0,18 ± 0,01	1,47 ± 0,2269	0,34 ± 0,04
C16:1*	0,24 ± 0,02	0,48 ± 0,01	1,71 ± 0,07	0,54 ± 0,0434	2,03 ± 0,13
C18:0	16,28 ±0,94	7,82 ± 1,33	5,33 ± 0,80	10,11 ± 0,0460	11,10 ± 1,06
C18:1	4,06 ±0,20	3,79 ± 0,15	3,09 ± 0,06	3,89 ± 0,0323	4,67 ± 0,48
11-C18:1	2,86 ±0,07	3,49 ± 0,12	3,37 ± 0,01	1,38 ± 0,0108	2,19 ± 0,05
C18:2 ω6	1,11 ±0,21	0,83 ± 0,23	0,85 ± 0,01	3,98 ± 0,0380	1,85 ± 0,22
C18:3 ω6			1,77 ± 0,14	0,51 ± 0,0151	1,81 ± 0,06
C18:3 ω3				5,47 ± 0,0616	
C20:4 ω3				2,58 ± 0,0253	2,77 ± 0,10
C20:4 ω6	2,52 ± 0,50	4,14 ± 0,14	2,59 ± 0,04	1,31 ± 0,0961	2,66 ± 0,08
C20:5	34,68± 0,84	44,16 ± 1,44	45,50 ± 0,97	40,24 ± 1,07	35,61 ± 0,65
Ratio ω6/ω3				0,72	2,27

A comparison between the samples from the same period of the year (wild collected at 1/02/2017 and sample collected from IMTA tank on 24/01/2017) was done. In the IMTA sample it was detected a different fatty acid (C18:3ω3) that it was not detected in the other samples. Also, the values of C20:5 was higher in the sample from the IMTA system in contrast to C16:0 that was lower (Fig. 9).

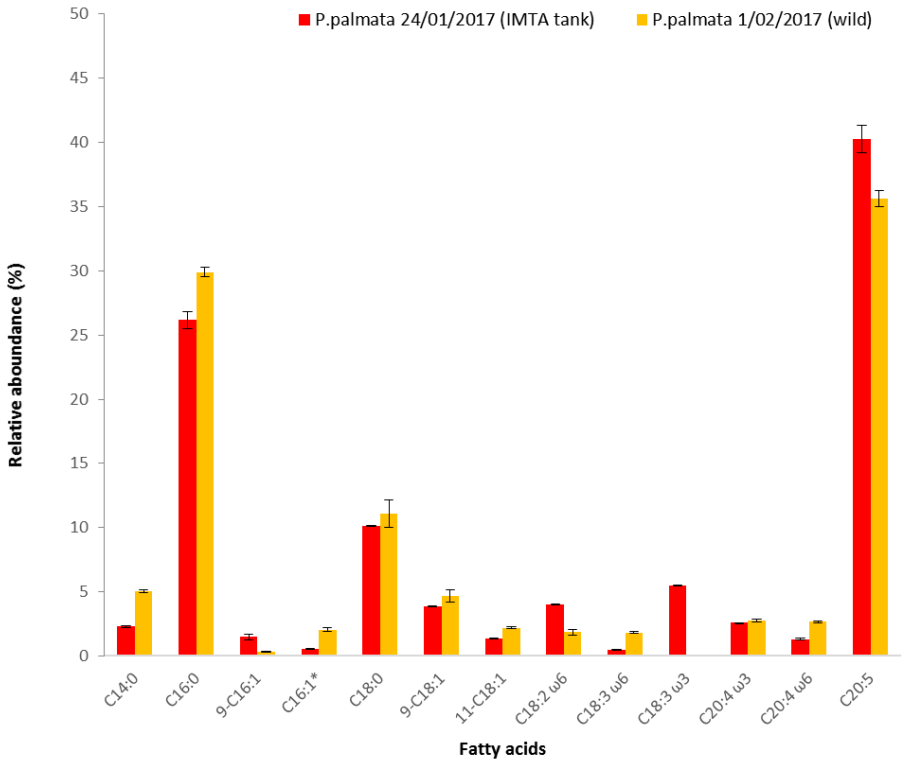

Fig. 9. Relative abundance of fatty acids in the samples collected from the IMTA tank on 24/01/2017 and the sample collected from the wild on 1/02/2017 expressed in %. Vertical lines represent the standard error. (n=3)

3.3.3 Ash content

The ash content varies between 14,5 ±0,67 % in the wild sample to 24,2 ± 0,86 % in the sample collected at 24/01/2016 from IMTA tank (Fig. 10).

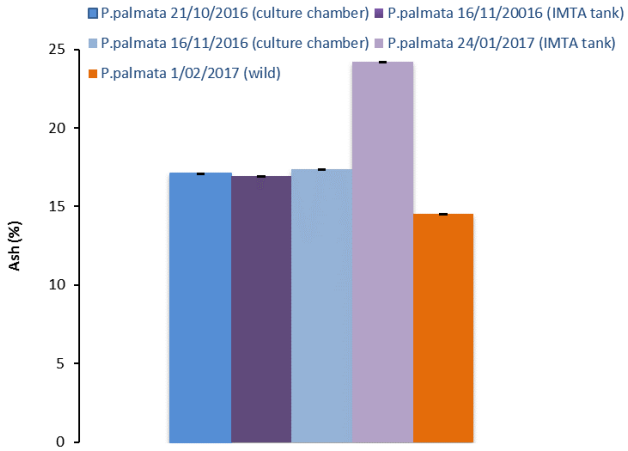

Fig. 10. Ash percentage of the samples analysed. Vertical lines represent the standard error. (n=3)

3.3.4 Carbohydrates content

Values of carbohydrates varies in the opposite way as the ashes, oscillating between 55,52% in the sample collected at 24/01/2016 from IMTA tank and 67,56 % in the wild sample (Fig. 11).

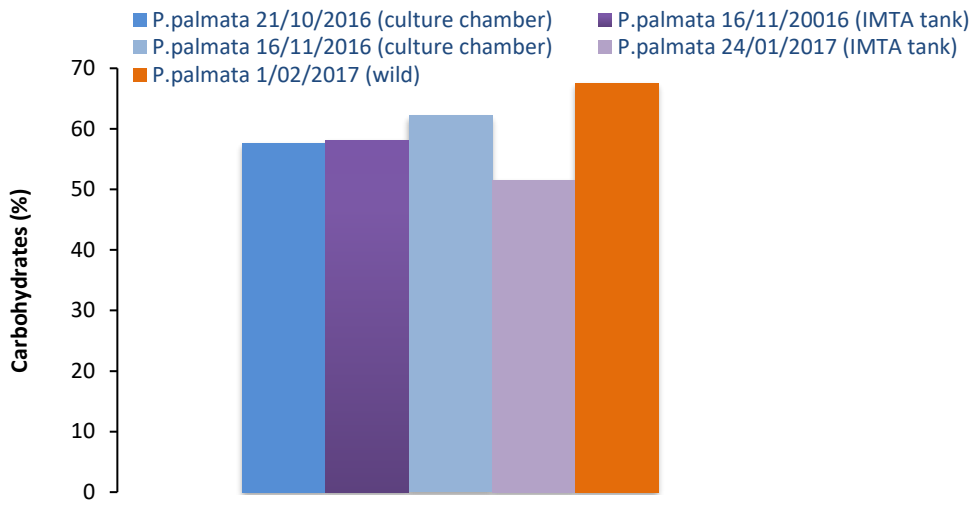

Fig. 11. Carbohydrates percentage of the samples analysed.

4.Discussion:

At the beginning of the experiment, two 20-L containers were cultured in the culture chamber. The culture period began with 540 g in one container and 360 g in the other, transferred to the outer tanks at two different times. After 87 days and from this initial inoculum material, 2908 g were obtained. Optimum stocking density in terms of productivity was 2 kg/ m^{-2} (Morgan et al., 1980). The average growth rate at lower density (1 kg/ m^{-2}) was higher than the rest of the cultures (Kim et al., 2013).

Lipid content has been described to be very low in seaweeds, ranging from 1 to 5% of dry matter and varies strongly between species, but their PUFA content can be as high as that of land plants or even higher (Schmid et al., 2014). High proportion of PUFA was detected in *Palmaria palmata* with 49,8 % (Schmid et al., 2014). The mains PUFA observed were C16:0, C18:0 and C20:5. The ratio of omega-6/omega-3 fatty acids was between 1 and 2 in contrast to (Schmid et al., 2014) were the lowest ratio observed in *Palmaria palmata* was 0,04 due to high amounts of 20:5 n-3 present.

One of the most interesting observations of this study was that the highest relative concentration of PUFAs in the red seaweed *Palmaria palmata* was observed for eicosapentaenoic acid (C20:5, EPA) accounting for 45% of the total fatty acid content in the sample from the culture chamber where the conditions of temperature of light were controlled. EPA is a very important n-3 FA in fish oil (Van Ginneken et al., 2011)

Although temperature represents one of the possible factors that can influence total lipid and fatty acid composition, and particularly PUFA levels, in macroalgae, it is likely that changes in other abiotic factors (for example: light, salinity, nutrients) and interactions between such factors have also contributed towards the observed variations (Schmid et al., 2014); additionally, the presence of different vegetative and reproductive stages at different times of the year when algae were collected requires further, more detailed investigation.

Different studies have shown that maximum values of proteins were found when maximum nutrients were available (Galland-Irmouli et al., 1999). In the present study, comparing the samples from the same period of the year, the one from IMTA tank has higher percentage of protein (23,3 ± 0,290) that the one collected from the wild (17,3 ± 0,057), probably because of the difference in the nutrients of the water.

The ash content which ranged from 14,5 ±0,676 % to 24,2 ± 0,864% were considerably similar than the 21.3–22.8% reported by Wong et al., (2000) but lower than the 20.6-39.3% reported by (Rupérez, 2002). The ash content of seaweeds vary between species, between geographical locations and between seasons (Sánchez-Machado et al., 2004)

The amount of carbohydrates oscillates between 55,52 and 67,56 %. These results were different than the ones from Morrissey et al., (2001) were the carbohydrates content varies between from 46 to 50 %.

5. Conclusions:

Although *Palmaria palmata* is a very important species because of its application for human consumption and extracts of high value, its possibilities for cultivation are limited because of its low temperature tolerance range. During the experimental period, it was possible to grow *Palmaria palmata* in tanks under the IMTA approach (high nutrients and tank control) in sustainable way with relative growth rates from 1,02 to 2,85 % d^{-1} and yield from 434,69 to 1270,39 g dw m^{-2} d^{-1}

Under these conditions, more studies should be carried out to obtain optimal values for seaweed density in cultivation tanks.

Proximal composition of *Palmaria palmata* grown in tanks with high nutrients (mainly N and P) shows high protein and lipid content in contrast to the sample collected from the coast (wild), where the amount of carbohydrates was higher than the samples from IMTA system. Also, a different fatty acid was detected (C18:3ω3) in the sample from IMTA system, that was not detected in the sample from the wild. More samples should be analysed to carry out statistical analyses and minimize the error.

6.Annexes:

IMTA: Integrated Multi-Trophic Aquaculture

PUFAs: Polyunsaturated Fatty Acids

EPA: Eicosapentaenoic acid

FAMEs: Fatty acid methyl esters

RGR: Relative Growth Rate

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8.Webgraphy:

http://people.umass.edu/~mcclemen/581Ash&Minerals.html

• Descripción detallada de las actividades desarrolladas durante la realización del TFT

Para el cultivo de *Palmaria palmata* era necesario medir la temperatura y salinidad, purgar las salidas del agua y ajustar flujos de renovación. Cada semana o cada 15 días se vacían los tanques para pesar nuestra alga y poder calcular tasas de crecimiento, productividad, etc. Después de pesarlas los tanques se vuelven a inocular ajustando las densidades y cambiando la capacidad del tanque si es necesario.

Con respecto al análisis químico realizado en la Universidad de Aveiro de las distintas muestras fue necesario seguir los protocolos de extracción y análisis detallados a lo largo del TFT.

• Formación recibida (cursos, programas informáticos, etc.)

Durante mi estancia en ALGAplus recibí por parte de los empleados las directrices para poder trabajar y realizar las actividades de la empresa. En la Universidad de Aveiro, donde realicé los análisis químicos, recibí por parte de los profesionales del laboratorio de lipidómica los protocolos necesarios para realizar las extracciones, así como la utilización de programas informáticos para la identificación de los ácidos grasos.

• Nivel de integración e implicación dentro del departamento y relaciones con el personal.

Mi nivel de integración e implicación ha sido muy bueno tanto en la empresa ALGAplus como en la Universidad de Aveiro donde me recibieron y trataron como una más del equipo. Mi especial agradecimiento a doña Elisabete Costa que me guio y ayudó en todos los análisis realizados en la universidad, haciendo incluso horas extras para ayudarme con mi trabajo. También estoy muy agradecida con los directores de ALGAplus, el Dr. Rui Pereira y a la Dra. Helena Abreu que me han dado la oportunidad de hacer prácticas en su empresa, así como a la Dra. Maria Rosário Domingues por permitirme unirme a su equipo de lipidómica en la universidad.

• Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

Los aspectos positivos de la realización de este TFT han sido poder trabajar con una empresa y aprender su funcionamiento y por otro lado poder entrar en un grupo de investigación de otra universidad y otro país en el cual he aprendido muchísimo de un área en la que no había profundizado nunca.

Un aspecto negativo es la desorganización acerca de la planificación con mi TFT al llegar a la empresa, también al ser la primera vez que va un alumno con estas características entiendo que es muy complicado adaptarse a nuestro sistema.

• Valoración personal del aprendizaje conseguido a lo largo del TFT.

Estoy muy contenta con todos los conocimientos que me llevo, tanto a nivel de trabajo en una empresa, gracias a la cual ya se cómo sería incorporarme al mundo laboral en el área que me gusta, como en un centro de investigación, donde me llevo muchos conocimientos que antes no tenía sobre todo de la parte de lípidos. Recomiendo a todos los alumnos que no tengan miedo a salir y hacer las prácticas y el TFT en otro país ya que te llevas una experiencia y unos conocimientos únicos.