TÍTULO

EFECTO DE LA RADIACIÓN UV Y NITRATO EN LA FOTOSÍNTESIS Y PRODUCCIÓN DE COMPUESTOS BIOACTIVOS EN EL ALGA EXÓTICA INVASORA RUGULOPTERYX OKAMURAE (OCHROPHYTA)

EFFECT OF UV RADIATION AND NITRATE ON PHOTOSYNTHESIS AND PRODUCTION OF BIOACTIVE COMPOUNDS IN THE INVASIVE EXOTIC ALGA RUGULOPTERYX OKAMURAE (OCHROPHYTA)

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En Málaga, a 3 de diciembre de 2021.

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RESUMEN

Rugulopteryx okamurae (Ochrophyta) es un alga parda de origen asiático que desde el año 2015 está invadiendo las aguas costeras de Andalucía. Es un alga con gran plasticidad fisiológica y morfológica y que crece tanto en aguas superficiales, expuestas a alta radiación e hidrodinamismo, hasta 30-40 m de profundidad en ambiente esciáfilo. En este TFM se aplica un diseño experimental bifactorial en el que se estudia la influencia de la concentración de nitrato (60, 480 y 900 μM) y radiación fotosintética (PAR) y PAR+UV+Azul sobre la actividad fotosintética y acumulación de compuestos bioactivos. Se evalúa la composición bioquímica de compuestos de metabolismo primario (proteínas, pigmentos fotosintéticos, lípidos y carbohidratos) y secundario (polifenoles) y se valora su capacidad antioxidante y fotoprotectora y su aplicación en productos cosmecéuticos. El mayor conocimiento ecofisiológico de esta especie puede contribuir al diseño de estrategias y programas en el marco de plan de gestión y control de la invasión por *Ruguloteryx okamurae* del ecosistema costero del Mar Mediterráneo. En este trabajo se ha observado una influencia de la radiación UV en la producción de lípidos y carbohidratos, mientras que el nitrógeno ha afectado positivamente en la obtención de proteínas.

Palabras clave: Antioxidantes, Aplicaciones cosmeceúticas, Especie exótica invasora, Fotoprotectores, Fotosíntesis, *Rugulopteryx okamurae*.

Effect of UV radiation and nitrate on photosynthesis and production of bioactive compounds in the invasive exotic alga *Rugulopteryx okamurae* (Ochrophyta)

ABSTRACT

Rugulopteryx okamurae (Ochrophyta) is a brown algae of Asiatic origin that since 2015 has been invading the coastal waters of Andalusia. It is an alga with great physiological and morphological plasticity and that grows in surface waters, exposed to high radiation and hydrodynamics, up to 30-40 m deep in a shaded environment. In this TFM, a bifactorial design is applied in which the influence of the concentration of nutrients and photosynthetic radiation (PAR) and PAR+UV+Blue on the photosynthetic activity and accumulation of bioactive compounds is studied. The biochemical composition of compounds of primary metabolism (proteins, photosynthetic pigments, lipids and carbohydrates) and secondary (polyphenols) are evaluated and their antioxidant and photoprotective capacity and their application in cosmeceutical products are evaluated. The increase in the ecophysiological knowledge of this species can contribute to the design of strategies and programs within the framework of the management and control plan of the invasion by *R. okamurae* of the coastal ecosystem of the Mediterranean Sea. In this work an influence of UV radiation has been observed in the production of lipids and carbohydrates, while nitrogen has positively affected the production of proteins.

Key words: Antioxidants, Cosmeceutical applications, Invasive exotic species, Photoprotectors, Photosynthesis, *Rugulopteryx okamurae.*

1. Introduction

Marine ecosystems are highly susceptible to rapid changes throughout the world. The effects of climate change threaten to destroy the stability of these ecosystems and the species that inhabit them globally. These effects include rising temperatures and acidification of the oceans due to the absorption of greenhouse gases, as well as human activities such as pollution from the dumping of garbage or agricultural residues.

In addition to these factors, there is the growing threat of the proliferation of invasive species: those introduced deliberately or unintentionally in regions other than their original habitat, being the first cause the introduction by maritime transport. Globalization has led to a reduction in the costs of maritime transport and, with this, the movement of species around the world has been facilitated, especially through ballast water loaded and unloaded by ships in ports (Elçiçek et al., 2013).

Rugulopteryx okamurae (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim (Dictyotaceae, Ochrophyta) is a brown macroalga native to the north-western Pacific (Huang, 1994; De Clerck et al., 2006) that was identified and cited in 2015 by the first time in the coasts of the Strait of Gibraltar (Ceuta and Tarifa) (Altamirano et al., 2016; Ocaña et al. 2016; El Aamri et al., 2018; García Gómez et al., 2021). Previously in the Mediterranean, it was cited by Verlaque (2009) on the French Mediterranean coast, probably introduced with the Japanese oyster *Crassostrea gigas* in the coastal lagoon of Thau.

The Strait of Gibraltar is the link between the Mediterranean Sea and the Atlantic Ocean, making it an area of great ecological interest. That is why it becomes a point of convergence of species from both territories, in addition to being a protected area with the figure of a Natural Park. In short, it is an area of great commercial importance, being one of the enclaves with the most maritime traffic in the world (Endrina et al., 2018), which makes it a vulnerable area to the accidental introduction of invasive species.

R. okamurae has experienced an unusual and unprecedented growth on the coasts of the Strait of Gibraltar and Andalusia in recent years. Just one year after its appearance in Ceuta, more than 5000 tons of algae biomass were removed upstream from the IAS (Institute of Sustainable Agriculture) by the beach cleaning machines in this city (Ocaña et al. 2016; García-Gómez et al. 2018). The expansion along the entire coastline occupying the coasts of Malaga, Granada, Huelva and Almeria has only taken 4 years,

thus affecting very relevant areas of great ecological value included in the Natura 2000 Network.

The most probable vectors of its introduction, from its native area of the Pacific, are ballast waters (Rosas-Guerrero et al. 2018), one of the main vectors of introduction of exotic marine species (Naylor et al. 2001).

The invasion of *R. okamurae* along the coast of Andalusia is causing a big environmental impact, as it alters the marine ecosystem and also affects the subtidal and intertidal biodiversity, with quantitative evidence of its effects on corals (Sempere-Valverde et al. 2021). On the other hand, it also implies a problem at a socioeconomic level in activities like fishing and tourism (Navarro-Barranco et al. 2019; García-Gómez et al. 2020). Landscape and human wellness are compromised because of the seaweed washed up on the beach and the unpleasant smell is produced due to the organic material decomposition. Regarding the economic impact, the expenses in the management of the beaches and the fishing activities exceed one million euros per year (Altamirano et al. 2016). Considering all these facts, it is crucial to advance in its management to control its expansion.

This has already happened with the macroalga *Asparagopsis taxiformis*, a red alga that has invaded the Andalusian coasts and displaced multiple native species (Mancuso et al., 2021), and a similar case has been reported in the Mexican Caribbean coast with *Sargassum* spp. (Van Tussenbroek et al. 2017).

There has been a growing interest in the study of natural products in recent years, due to their ecological character and their usefulness in humans (Hernández-Carmona et al. 2012). Different local collectives affected by the invasion of *R. okamurae* have proposed to treat this macroalgae not as a waste but as a valuable raw material for cosmetic products, dermatology, composting for natural fertilizers for plants, etc. Recently it has been proposed to manufacture shoe insoles (https://www.lavozdigital.es/cadiz/provincia/lvdi-utilizan-alga-invasora-para-fabricar-plantillas-zapatos-y-abono-ecologico-202108160904_noticia.html/ accessed 28 November 2021) and to produce methane by means of anaerobic codigestion with solid oil mill waste (Lama et al., 2021). This supposes a biotechnological valorization of the invasive algae to find sustainable uses and exploitation.

In order to predict what its expansion will be in order to act in advance and develop plans and strategies to manage and stop the invasion, the need arises to understand the

physiology of this alga by studying the multiple factors that may have allowed its rapid expansion. along the coasts of Andalusia and the Strait. Temperature, light, as well as the concentration of nutrients in the environment, are variables that could be involved as environmental factors in the spread of this invasive algae (Huesa-Cerdán et al. 2020). In this work, two of these factors have been investigated: light and nutrients.

Taking all this into account, a bifactorial experiment with different nitrate concentrations (60µM, 480µM and 900µM) and two light treatments - PAR (400-700 nm) and PAR + UVR + Blue (280-400 nm) - has been carried out to evaluate the possible effect of ultraviolet light and the nutrient concentrations, as well as the interaction between them, on photosynthesis and the production of bioactive compounds with potential interest in biotechnology.

The hypothesis of this work is that *Rugulopteryx okamurae* responds differently to different light treatments and nitrate concentrations, synthesizing more bioactive compounds under the effect of UV radiation at higher nitrate concentrations, treatments in which it would be more acclimated.

2. Objectives

The general objective of this work is to study the interactive effect between two environmental factors in the context of global change (nitrate and UV radiation) on photosynthetic activity and the accumulation of bioactive compounds such as phenols, lipids, proteins, etc. The antioxidant activity of the latter and the photoprotective capacity of the extracts will be evaluated with a view to the potential biotechnological application of the *Rugulopteryx okamurae* biomass.

To meet this general objective, the following specific objectives are proposed:

- Study the interactive effects of nitrogen and UVR on growth, photosynthesis and the ability to synthesize internal metabolites in *Rugulopteryx okamurae*.
- Examine the evolution of different photosynthetic parameters such as the maximum quantum yield (FSII) of photosystem II, the electron transport rate and the photosynthetic yield by performing Rapid Light Curves (RLC), which will serve to characterize the photosynthetic response of this alga in response to the environmental conditions studied factors.

- Analyze the concentration of biomolecules and compounds that can be affected by variation in the concentration of nutrients and light, such as carbohydrates, lipids, proteins, photosynthetic pigments, phenolic compounds and the antioxidant capacity that give all of them.
- Elaborate a cosmetic product from extracts obtained from *Rugulopteryx okamurae* and other ingredients used in natural cosmetics and evaluate it photoprotection capacity.

3. Materials and methods

3.1. Biological material

Rugulopteryx okamurae (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim in I.K.Hwang, W.J.Lee, H.S.Kim (Dictyotales, Ochrophyta), native to the northwestern Pacific (Huang, 1994), was identified and cited for the first time in the Strait of Gibraltar (Ceuta and Tarifa) from seaweed washed ashore (Altamirano et al., 2016) although the first citation in Europe was on 2002 in Thau Lagoon (France) (Verlaque et al., 2009). The route of entry of the brown alga to the Strait is unknown, although probably they were transported in ballast waters of vessels from Asia region in the harbours of Algeciras and Ceuta (Spain) and Tangermed (Marroco). The establishment has occurred in an expansive and overwhelming way, generating an unprecedented visual and ecological impact on these shores (García Gómez et al., 2020).

The order of the Ministry for ecological transition and demographic challenge of Spanish government (TED/1126/2020 of 20 November, 2020) approved the inclusion of *R. okamurae* in the list of invasive exotic species (BOE 314 of 1 December, 2020).

On June 10, 2021, an administrative authorization was received from the Secretary of State for the Environment for the extraction of the invasive exotic algae and the monitoring of its populations on the Spanish coasts for the research project "Photoprotectors of algae: cosmeceuticals compatible with the ocean (FACCO)" of the call for aid to R + D + i projects of the FEDER Andalusia Operational Program 2014-2020 (Expte PNE/21/187/INV) (Appendix-3).

The taxonomical classification of *Rugulopteryx okamurae* is as follows (algaebase.org): Empire: Eukaryota, kingdom: Chromista, phylum: Ochrophyta, class: Phaeophyceae,

subclass: Dictyotophycidae, order: Dictyotales, family: Dictyotaceae, tribe: Dictyoteae and genus: Rugulopteryx

Sinonimias: *Dictyota okamurae* (E.Y. Dawson) Hörnig, R.Schnetter & Prud'homme van Reine 1993; *Dictyota marginata* Okamura 1913; *Dilophus marginatus* (Okamura) Okamura 1915; *Dilophus okamurae* E.Y.Dawson 1950.

Rugulopteryx okamurae is a cryptic species that it can be confused with species of the genus Dictyota or Taonia. The most reliable morphological characteristic to differentiate the Dictyota and *Rugulopteryx* genera is the number of basal cells of the sporangium (one in Dictyota and two in Rugulopteryx) (De Clerck et al., 2006; Hwang et al., 2009).

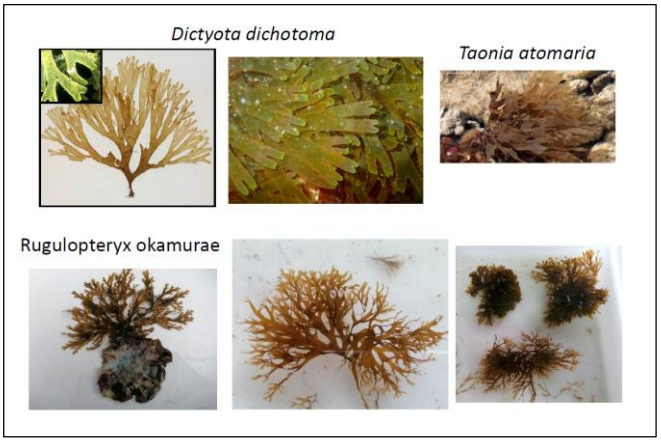

Figure.3.1. Photographs of *Dictyota dichotoma*, *Taonia atomaria* and *Rugulopteryx okamurae* showing its high morphological diversity

At the macroscopic level, individuals of *Rugulopteryx okamurae* have a thallus 10-20 cm high and presents a high morphological variation, having described two main morphotypes and one intermediate (Sun et al., 2006). The thick of thallus (sporophyte) 120-180 μm and the wide about 5-10 mm wide. *R.okamurae* has a multilayer medulla on the margins of the thallus (Okamura, 1913). The dimension of intermediate part of the thallus (Sporophyte or gametophyte) is 2 - 4 cm wide and less than 100 μm thick

Finally, the basal part of the thallus presents the characteristics of the thick morphotype and the apical part of the fine one.

Reproduction is mainly asexual (Hwang et al., 2009) and is carried out by means of sporangia without involvement located on both sides and along the entire surface of the thallus, except for the marginal and apical parts (Verlaque et al. 2009). Sporophytes

release spores in the summer and produce two types of spores: monospores and tetraspores. The monospores tend to develop in-situ forming propagules during the days prior to the release of the sporangia, while the tetraspores (monospores divided by mitosis) are released without germinating (Kajimura, 1992; Hwang et al., 2009).

3.2. Experimental design

Rugulopteryx okamurae was collected at La Caleta beach (Tarifa, Cádiz), on June 11th 2021 and transported to Malaga in refrigerated box. They were washed with seawater and small crustaceans, polychaetes and remaining fragments of *Asparagopsis taxiformis* were removed.

The experiment was set up in a thermostatic culture room, whose fixed temperature was 22ºC. For the preincubation period, the samples were in the culture chamber in UV transparent cylindrical vessel of polyvinyl methacrylate with seawater for 3 days. A glass rod connected to an aeration system was inserted into each cylinder to avoid stagnation and thus keep the algae in motion and they were covered with cling film transparent to UV radiation.

Multifactorial experiment was developed using light and nutrient availability as factors. Two different light conditions were established, one using only Photosynthetic Active Radiation (PAR, λ=400-700 nm) and the other light treatment with PAR, Blue light (λ=400-450 nm) and Ultraviolet A radiation (λ=315-400 nm) (called PAB treatment). Three different concentration of nitrate were tested, 60µM, 480µM and 900µM as sodium nitrate with 30µM for sodium glycerophosphate.

The experiment was carried out in triplicate, thus in the room were housed 18 cylindrical vessel in total, 9 on the top shelf with only PAR light and 9 on the bottom shelf with PAR +Blue+UVR. The PAR light came from white light LED lamps (4500K) placed under and above the cylinders at an angle of approximately 45º emitting an irradiance of 300 µmol $m^{-2}s^{-1}$. The radiation emitted by the two white light LED lamps when they are lit is what will be called PAR^{+} from now on. For the other light factor, the same white lamps were placed and, in addition, a Blue+UVR lamp (lmax=365 nm) above the cylinders and a UVA radiation lamp (QPanel 340) just in front of the inclined white one, at the other end, emitting an irradiance of 330µmol $m^{-2}s^{-1}$ of PAR+Blue+UVR (called PAB) 7.95W·m^{-2} of UVA radiation and 27.6 W·m^{-2} of Blue+UVR. Light were measured with

a spectroradiometer (SphereOptics SMS-500). On each shelf a fan was placed on the left side pointing to the row of cylinders to avoid an increase in temperature due to the radiation emitted by the lamps.

The LED tube has 40 UVA LEDs (365 nm), 25 B LEDs (405 nm), 25 B LEDs (409 nm) and 30 B LEDs (450 nm). The spectrum of the Q panel is broader, with the maximum at 340 nm.

A 12:12 h light:dark photoperiod was used. The white LED lamps placed above the cylinders were turned on from 9:00 a.m. to 9:00 p.m. and the rest of the lights (white LED lamp placed under the cylinders, UVR and Blue LED) were turned on from 1:00 p.m. to 5:00 p.m., thus simulating the cycle of greater solar radiation that they would have in their natural habitat.

Figure. 3.2. Image of the 18 cylindrical vessel with the different light treatments (PAR above and PAB below) in the thermostatic culture room.

In the following table it is shown the daily dose of UVA, blue and PAR received by both light treatments.

Table 3.1. Dose ($kJ \cdot m^{-2} \cdot day^{-1}$) for light treatment: white LED irradiance (PAR) and white LED+Blue+UVR irradiance (PAB).

	Daily dose ($kJ \cdot m^{-2} \cdot day^{-1}$)	
	PAR	PAB
UV-A (320-399 nm)	0.86	54.71
BLUE (400-499 nm)	359.88	577.13
PAR (400-700 nm)	1690.56	1909.64
TOTAL	2051.3	2541.47

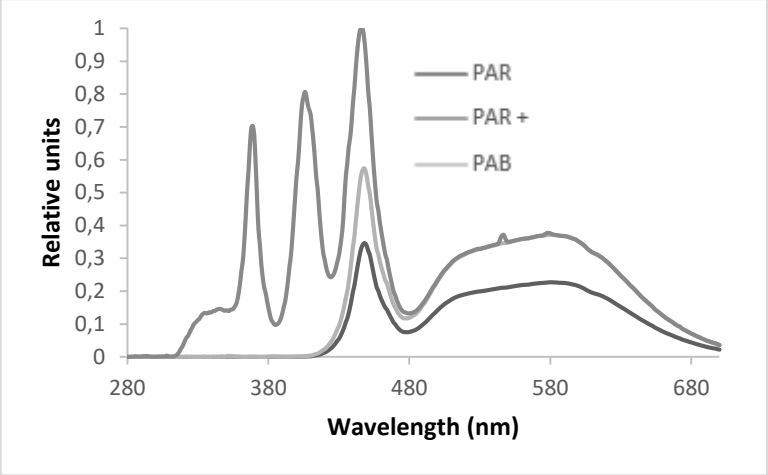

Figure 3.3. Normalized light spectrum for each light treatment in the experiment. Data are normalized for PAB between 0 and 1, and PAR and PAR^+ with respect the PAB treatment.

The different nutrient treatments were added through a modified Provasoli culture medium (Provasoli, 1963) for enriched seawater (Bold y Wynne, 1978). A Provasoli stock was prepared without adding the sodium nitrate, with a concentration of 30 µM of sodium glycerol phosphate. On the other hand, 3 stock of sodium nitrate of the 3 different concentrations (60µM, 480µM and 900µM) were prepared. For the experiment, 20 mL of the Provasoli stock and 5 mL of the corresponding NO_3^- stock were added to each cylinder (1 L seawater).

The experiment was conducted for 14 days, during which water samples (for determining nutrient concentration) were taken on day 7 and 14 and photosynthetic measurements were taken on days 0, 3, 7, 10 and 14. The temperature and pH of the water were monitored every 3 days in each cylinder using a glass rod thermometer and a portable pH/ORP-meter LAQUAct-PH110-K. In addition, an extra cylinder was put under the same conditions as the rest by introducing a data logger (HOBO MX Temp/Light (MX2202)) with a nut and a thread to monitor temperature.

The initial day of the experiment (day 0), the algae that were in the chamber during the acclimatization period were removed from the cylinders, mixed and the excess water was removed with paper. Three grams were weighed for each experimental replica and were introduced into the culture cylinders, previously washed and dried, this time with water prepared with the nitrate and phosphate ratios designated for the experiment. The rest of the biomass was designated as initial and was frozen in aluminium foil sachets.

The water was renewed using these same concentrations of nutrients on days 3, 6 and 10 of the experiment. For this, the cylinders were emptied, washed and dried before adding the culture medium again.

Once the experiment was finished, the *R. okamurae* samples were kept lyophilized until their analysis, except for photosynthesis, which was carried out in situ, and the water samples were kept frozen.

3. 3. Nutrients in seawater

10 mL of water were taken using syringe fitted with a filter of 25mm (Whatman Grade GF/C glass filters) from each of the replicates throughout the experiment and frozen in falcons. The water samples were passed through a biochemical analyzer (TECHNICON), to determine the concentration of nutrients. Nutrient consumption in the seawater was evaluated according to the method of Grasshoff et al. (1983). In this case, nutrients were analysed by segmented flow analyser (SFA) using a Seal Analytical autoanalyzer QuAAtro. The detection limits of inorganic nutrients were 0.05 µM for nitrates and phosphates. These data were assessed at the beginning the experiment, and at 3st, 7th, 10th and 14th experimental days of experiment. It was calculated the efficiency of nutrient uptake (%) per day.

3.4. Growth rate

On day 7 and 14 of the experiment, in addition to the photosynthetic measurements, the biomass of each cylinder was weighed. At day 7 the excess biomass after adjusting to the initial density of the experimental biomass (3 g $\cdot L^{-1}$), was stored in aluminum foil at -20ºC until the performance of the biochemical analysis.

The growth rates (% day^{-1}) were calculated using the initial fresh weight (3 grams) and the final fresh weight after 7 and 14 days), using the Yong formula (Yong et al., 2013):

$$Growth\ rate\ (\%\ day^{-1}) = \left[\left(\frac{Final\ mass}{Initial\ mass}\right)^{1/time} - 1\right] \cdot 100$$

3.5. Analysis of photosynthetic parameters by using *in vivo* Chlorophyll *a* fluorescence

In vivo chlorophyll *a* fluorescence associated with photosystem II (P680) using pulse amplitude modulated fluorimeters (PAM), is an indirect non-intrusive measurement (Yun Lee et al., 1997) and it was used to estimate photosynthetic rate and the physiological status of the algae.

This technique makes it possible to determine the quantum yields, the photosynthetic capacity and the amount of energy that is transferred to the electronic transport chain and that which is dissipated as heat during photosynthesis (Schreiber et al. 1986).

When a chlorophyll molecule, located in the chloroplasts, is excited by a photon, the light absorbed in the form of energy by photosystem II (P680) can be transferred to the reaction centers to be used photochemically, dissipated as heat and as fluorescence.

In the dark: the reaction centers are "open", quinone A (Q_A), which is a primary electron acceptor, is oxidized and therefore the reaction centers can photochemically reduce Q_A. If the sample was adapted to the dark before the application of the saturation pulse, the fluorescence yield will reach its true maximum (F_m).

The quantum yield is the efficiency with which the absorbed light is used for photochemical processes.

Optimal or maximal quantum yield (F_v/F_m), indicates the physiological state and photoinhibition for plants adapted to darkness and it is used as indicator of photoinhibition.

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

Where:

F_0: basal fluorescence

$F_{m:}$ maximum fluorescence

F_v: variable fluorescence

Effective quantum yield ($\Delta F/F_m{}'$) is an indicator of the light efficiency conditions for light adapted plants.

$$\frac{\Delta F}{F_m{}'} = \frac{(F_m{}' - F_t)}{F_m{}'}$$

Where:

F_t: intrinsic fluorescence in light

$F_m{}'$: maximum fluorescence

The measurement of effective quantum yield was carried out *in situ* on days 3, 7, 10 and 14 of the experiment at 8:30 a.m. after incubation of the samples in dark conditions overnight and at 3:00 p.m. Three saturation pulses were performed on the algae samples from each of the 18 cylinders with a MINI-PAM-II (Walz GmbH, Germany) fluorometer.

Rapid Light Curves (RLC) were performed at 9:30 a.m. using MINI-PAM-II (Walz GmbH, Germany) associated to the software WinControl-3. These curves were made by calculating the electron transport rate (ETR) for 12 increasing intensities of light radiation provided by an actinic light from the MINI-PAM II fluorimeter, incubating each one of them for 30 seconds. The first pulse was performed to obtain the value of F_v/F_m, after 15 minutes of incubation to adapt the algae to darkness.

The electron transport rate (ETR) is an estimator of the photosynthetic capacity or of the productivity, it is calculated as follow (Schreiber et al. 1997):

$$\text{ETR} = \frac{\Delta F}{F_m{}'} \cdot \text{EPAR} \cdot F_{II} \cdot \text{A} \qquad Units: \mu mol\ e^-\ m^{-2}\ s^{-1}$$

F_{II} = proportion of light absorbed by photosystem II i.e. 0.8 in brown algae according to Figueroa et al. (2014)

A = absorptance (proportion of absorbed light)

Absorptance was measured as reported by Figueroa et al. (2009), and is defined as 1-T-R, where T is the fraction of the incident irradiance that is transmitted through the tissue and R is the reflectance. It is calculated by measuring the incident light on the radiometer (E_0) and the incident light when the algae is on the slot, giving the transmitted irradiance (E_t) (Figueroa et al., 2009):

$$A = 1 - \frac{E_t}{E_0} \qquad (relative\ units)$$

The RLC parameters, maximum electron transport capacity (ETR_{max}), photosynthetic efficiency (α) and saturation irradiance (E_k), were obtained with the R program using a

script developed by Pablo Cobos Mateo. These parameters were calculated using a tangential fit based on the formula of Eilers and Peeters (1988):

$$P = \frac{E}{aE^2 + bE + c} \qquad P_{\max} = \frac{1}{b + 2\sqrt{ac}} \qquad initial slope \quad \alpha = \frac{1}{c}$$

Non-photochemical quenching (NPQ) is the expression of total energy dissipation and was calculated according to the ratio of both yield losses, which is similar to Stern-Volmer NPQ parameter (Bilger et al., 1995) as follows:

$$NPQ = \frac{(Fm - Fm)}{F\mathrm{m}}$$

After each saturating pulse during the RLCs, NPQ was calculated. In addition, NPQ versus irradiance relationships were determined. These curves were then fitted with R program to obtain the maximum NPQ values (NPQmax) using a tangential fit based on the formula of Eilers and Peeters (1988).

3.6. Elemental analysis (internal C, N, S)

Elemental analysis is a technique that quantitatively determines the total content of C, H, N and S present in samples of a very varied nature, both organic and inorganic, solid or liquid. For this, a LECO CHNS 932 elemental analyzer located in the Central Research Support Services (SCAI) of the University of Malaga was used. The technique is based on the complete and instantaneous oxidation of the sample through complete combustion under optimal conditions (950ºC to 1300ºC and atmosphere of pure oxygen). The different combustion products (CO_2 and N_2) are transported via carrier gas (He) through a reduction tube and then selectively separated in specific columns to be later thermally desorbed. Finally, the gases pass separately through a thermal conductivity detector that provides a signal proportional to the concentration of each of the individual components. EDTA (ethylenediaminetetraacetic acid) was used as a standard, a molecule whose carbon and nitrogen content (%) is well known.

The samples were supplied in powder by crushing them in a mortar. The results were expressed in mg · g^{-1} DW.

3.7. Photosynthetic pigments

For the analysis of photosynthetic pigments 20 mg of lyophilized biomass were homogenized in 1 ml of acetone 90% in mortar with sterilized sea sand. The acetone 90% was made up using a magnesium carbonate hydroxide saturated solution to remove any acid present. The samples were left overnight in the dark at 4 ° C and the next day they were centrifuged for 20 min at 4 ° C and 4000 rpm. The supernatant was taken and transferred to a new eppendorf tube, making up to 1 mL with 90% acetone (to avoid the extract being concentrated and to compensate for the part that could have evaporated). The absorption spectrum of each of the samples was measured in the Shimadzu UV Mini 1240 spectrophotometer between 400 and 750 nm.

$Chlorophylls\ a$ and $c1 + c2$ were determined according to Ritchie (2006, 2008) method. The formulas used to calculate the concentration were the following:

$$Chla\ =\ 11.47 \cdot\ (A_{664} - A_{750}) - 0{,}45 \cdot\ (A_{630} - A_{750})$$

$$Chl(c1 + c2) =\ 22.679 \cdot\ (A_{630} - A_{750}) - 3{,}404 \cdot\ (A_{664} - A_{750})$$

For carotenoids, the equation of Parsons & Strickland (1963) was used:

$$Carotenoids\ =\ 10 \cdot\ (A_{480} - A_{750})$$

In all the formulas expressed above, A_λ is the optical density of the extract at the determined wavelength. These concentrations, expressed in $\mu g \cdot ml^{-1}$, were divided by the weight of the analyzed sample and multiplied by the volume of extraction. The results were expressed in $mg\ of\ chlorophyll\ or\ carotenoids \cdot g\ dried\ weight^{-1}$ $(mg \cdot g\ DW^{-1})$ (Korbee, 2004).

3.8. Determination of total carbohydrates, soluble protein and total lipids

Total carbohydrates

The Dubois method (Dubois et al, 1956) was used for carbohydrate quantification. By this assay, hexoses, disaccharides, oligosaccharides, polysaccharides and methylated derivatives having a free or potentially free reducing group are quantified. The method does not measure glucosamines and galactosamines, but includes pentoses and methylpentoses (Kochert, 1978). 5 mg of lyophilized biomass from each replica were

weighed into glass tubes and 5 mL of 1 M sulfuric acid (H_2SO_4) were added. These tubes were placed in a water bath at 100 ° C for one hour. They were then removed from the bath and allowed to cool to room temperature. The cooled tubes were centrifuged it was centrifuged at 4000 rpm for 10 min at 15 ° C. Subsequently, 1 mL of supernatant was separated with a glass Pasteur pipet. 500 µL of supernatant was mixed with 500 µL of 1 M sulfuric acid to dilute the sample. Then 1 mL of 5% phenol was added, allowed to stand for 40 minutes, and 5 mL of concentrated sulfuric acid was slowly added. The blank was prepared in the same way, but with 1 mL of 1 M sulfuric acid. The samples were allowed to cool to room temperature, and the absorbance at 485 nm was measured in the spectrophotometer. Different glucose concentrations will be used for the standard curve. The results where expressed in $mg \cdot g^{-1}$ DW.

Soluble protein content

The quantification of the total soluble protein content of the samples was carried out following the Bradford method (Bradford, 1976) using the commercial Bio-Rad solution. The same extract prepared for the ABTS method was used. 780 µl of phosphate buffer, 200 µl Bio-Rad and 20 µl of sample were mixed. It was stirred and waited for 15 min to measure the absorbance at 595 nm against a blank formed by phosphate buffer added to the Bio-Rad. For quantification, a standard curve was elaborated using *Bovine Serum Albumin (BSA)* (Sigma-Aldrich). The curve was prepared in a concentration range between 0 and 20 µg BSA/ ml. The results were expressed in mg soluble protein·g dried weight^{-1} (mg·g DW^{-1}).

Total lipids

The total lipids content was determined using the Folch method (Folch et al., 1956). For the extraction a chloroform-methanol mixture 2:1 by volume was used, containing a 0.01% of BHT (*Butylated hydroxytoluene)* (Sigma-Aldrich). 50 mg of lyophilized algae was weight in a "A" tube and 5 ml of chloroform-methanol (2:1) and 0.01% BHT was added. It was weighed into glass tubes and labelled with a pencil and sticker because of the organic solvents. The samples were homogenized in UltraTurrax T-10 Basic (Sigma-Aldrich) for 5 minutes (putting the tube on ice so that the sample did not heat up and prevented it from oxidizing) and then rinsed with 5ml of chloroform-methanol. After

each sample change, the stem was cleaned with 5 ml of chloroform-methanol. 2 ml of 0.88% KCl (Sigma-Aldrich) was added to each tube. Then, it was centrifuged for 5 minutes at 2000 rpm to separate into two phases (salt + methanol in the upper part and lipids + chloroform in the lower part).

The upper phase was discarded to organic residues and the lower phase was filtered in a tube B previously weighed. The samples were filtered using glass funnels and paper filters. A tip of anhydrous sodium sulphate (Na_2SO_4) (Sigma-Aldrich) was added to the filter and soaked with trichloromethane ($CHCl_3$) before passing the sample so that lipids were not retained. The filter was cleaned with $CHCl_3$. The tubes with the filtered samples were kept in the freezer for evaporation the next day. The salt layer was removed with a glass pipette when removing the samples from the freezer. Finally, the frozen samples were evaporated to dryness with N_2 in the extraction chamber. After evaporation was complete, the tubes with the samples were weighed. The percentage of lipids was calculated using the following formula, where the lipid mass corresponds to the difference between the mass of the tubes with lipids and that of the empty tubes:

$$\%\, Lípids\ = \frac{g\ Lipids}{g\ Sample} \cdot 100$$

3.9. Phenolic compounds

Phenolic compounds were determined according to Folin & Ciocalteau (1927). The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate, used for the determination of phenolic and polyphenolic compounds (Singleton et al. 1999). 20 mg of lyophilized algae were crushed in a mortar with sterilized sea sand with 80% methanol as reagent (the process was done on ice). The extracts were collected in eppendorfs of 1.5 ml and left overnight in the dark at 4°C. After that period, 100 µl of the extract, 700 µl of distilled water and 50 µl of Folin-Ciocalteu reagent (Sigma-Aldrich) were added, stirred and finally 150 µl of 20% Na2CO3 were added. The samples were shaken again and the samples were incubated for 2 h in the dark at 4 ° C. The absorbance at 760 nm was measured.

The Folin-Ciocalteu method tends to interfere with proteins, so PVPP (Polyvinylpolypyrrolidone) (Sigma-Aldrich) was used for a more accurate measurement of phenols, an insoluble polymer that binds to phlorotannins and precipitates them. Phenols were measured without adding PVPP and after adding PVPP to the extract. 5mg

PVPP was added to the extract, stirred several times, it was centrifuged and the same Folin-Ciocalteu method indicated above was run again with the new supernatant. To calculate the "real" amount of phenols, the amount measured with PVPP was subtracted from the amount obtained without PVPP, thus obtaining the amount of phenols that have precipitated. The phenolic content was calculated as standard curve of gallic acid from 1 to 20 µg/ml. The results were expressed in $mg \cdot g^{-1}$ DW.

3.10. Antioxidant capacity

3.10.1. ABTS method

Antioxidant capacity according to the ABTS method was determined as described by Re et al (1999). 20 mg of lyophilized biomass were homogenized in 1 ml of phosphate buffer (0.1 M, pH 7.0) in mortar with sea sand in an ice box and left to extract overnight in the dark at 4°C. The radical $ABTS^+$ is formed by the reaction between ABTS (2,2`-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (Sigma-Aldrich) y potassium persulfate ($K_2S_2O_8$) (Sigma-Aldrich). It was produced by adding 38.41mg of ABTS (7mM) to 10 ml of phosphate buffer (0.1M, pH = 6.5), shake it vigorously and add 6.62mg of potassium persulfate (2.45mM), shake it again and store it in darkness.

Next day, the extract was centrifuged at 4000 rpm for 10 min and the supernatant was used for the assay. The $ABTS^+$ solution was diluted to an initial absorbance of approximately 0.75 at the measurement wavelength (727nm). In a 1 ml cuvette, 950µl of the $ABTS^+$ solution and 50µl of the algal sample were added. The initial absorbance was measured before adding the sample, replacing the 50 µl of sample with phosphate buffer, and once added, the absorbance was measured after 8 min of reaction (final absorbance). Blank was done by only using the extraction solvent.

The calculation of the antioxidant activity, expressed as a percentage (%AA), was carried out using the following formula:

$$AA\% = \frac{(Initial\ absorbance - Final\ absorbance)}{Initial\ absorbance} \cdot 100$$

A standard curve of 0 to 5 µg/ml of Trolox was prepared, starting from a stock solution of 1mg/ml with ethanol. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich) is a highly oxidizing compound, analogous to vitamin E and that dissolves in

ethanol. The units were converted to Trolox equivalents, therefore the results were expressed in µmol of Trolox equivalents·g dried weight^{-1} (µmol TE·g DW^{-1}).

3.10.2. DPPH method

Antioxidant capacity of macroalgae extracts were determined using the method based on the reduction of the stable free radical DPPH, according to the method of Blois (1958). Free radicals are chemical species that are characterized by having one or more unpaired electrons. The 1,1-diphenyl-2-picrylhydracil or DPPH free radical is capable of reacting with antioxidant compounds through a process characterized by the transfer of a hydrogen atom provided by the antioxidant agent. Antioxidant substances of the algae extracts react with DPPH and the reduction of the reagent is followed by measuring the decrease in absorbance at 517nm. The DPPH radical doesn't need to be generated, but is obtained directly by dissolving it in an organic solvent such as methanol. In contradistinction to ABTS method, the DPPH, when is dissolved in organic solvent, evaluates the antioxidant capacity of the compounds that dissolve in those organics (phenols, chlorophylls, carotenes, etc.) and not the antioxidant capacity of the compounds that only dissolve in water or phosphate buffer. The same extract prepared for the phenolic compounds method was used. It was centrifuged at 4000 g for 15 min at 4ºC, the supernatant being the extract. A solution of DPPH (Sigma-Aldrich) at 1.27 mM in 90% methanol was prepared, keeping it in ice and in the dark for its optimal lifetime of 4 h. 150 µl of 1.27 mM DPPH, 1.35 ml of 90% methanol and 150 µl of seaweed extract were added, stirred and were incubated in the dark for 30 min at room temperature.

Before add DPPH and 90% methanol, dilutions $\frac{1}{1}, \frac{1}{4}, \frac{1}{8}$ and $\frac{1}{16}$ were made to get a color gamut of the reagent and calculate the EC_{50}. A DPPH standard curve was prepared with concentrations between 0 and 100 µM with 90% methanol and the DPPH stock.

It was also calculated the µmol of Trolox equivalents·g dried weight^{-1} (µmol TE·g DW^{-1}) for DPPH method. For this, a standard curve of 0 to 15 µg/ml of Trolox was prepared.

3.11. Elaboration of a cosmeceutical product with photoprotective and antioxidant capacity from *R. okamurae* extract

Four cosmetic creams were designed from a base cream (B) composed of the basic components of a standard cosmetic (control +) adding the algal extract. Two aqueous extracts (10 and 15%) and two hydroalcoholic extracts (10 and 15%) were prepared. For this, the algae were crushed before adding them to the solvent and heated in a water bath for 6 hours at 45ºC. They were then filtered and centrifuged at 3000 rpm for 4 minutes. The algae extract and the control cream were mixed using an Unguator 2100 model emulsifier (GAKO International GmbH, Munich, Germany). All formulations were mixed at 750 rpm for 2 min.

3.12. Determination of *in vitro* Sun Protection Factor (SPF*in vitro*)

The sun protection factor was determined for the pure extracts and for the creams according to the *in vitro* method COLIPA (2011). First, the UV lamp (Qpanel 340, USA) was placed at a certain height with respect to the table, where the samples would later go to irradiate, and the spectral irradiance was measured with a Multidiode spectroradiometer (SphereOptics SMS-500). UV irradiance had to be under the following specifications:

Table 3.2. Light source specifications.

Total UV irradiance (290 to 400 nm)	50 – 140 $W \cdot m^{-2}$
Irradiance ratio of $UVA_{(320 \text{ to } 400 \text{ nm})}$ to $UVB_{(290 \text{ to } 320 \text{ nm})}$	8-22

The product was distributed homogeneously on the PMMA plates over the entire surface without exerting much pressure, with a finger fitted with the inner part of a latex glove, for approximately 30 seconds. For each cosmetic preparation, three plates were prepared, which were kept protected from exposure to light and at room temperature (≈20°C) for 15 minutes before their measurement. The transmission (%) of UVR through the sample was measured at a spectral range of 290 to 400 nm, at intervals of 1 nm and at 4 different sites on the plate. The blank was prepared with glycerin (1.3 $mg \cdot cm^{-2}$) using commercial PMMA plates according to the physicochemical properties described by Pissavini et al. (2009). The Sun Protection Factor (SPF) was calculated with the data obtained by the spectrophotometer, for the UVR spectral range between 290-400 nm:

$$SPF_{in\ vitro} = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * 10^{-A_0(\lambda)} * d\lambda}$$

where: E = Erythema action spectrum (CIE- 1987); I= Spectral irradiance of the UV source spectrophotometer with integrate sphere); A= Mean monochromatic absorbance measurements per plate of the test product layer *before* UV exposure and d= Wavelength step (1 nm).

3.13. Statistical analysis

Analysis of variance (ANOVA) were carried out, taking days (7 and 14), nutrient concentration (60µM, 480µM and 900µM) and light (PAR and PAR + UV) as categorical factors (Appendix-1). Cochran test was used to evaluate the homogeneity of variances. After significant differences ($p < 0.05$), post-hoc Student-Newman-Keuls test was carried out (Underwood, 1997). Pearson's correlations were also determined for all the variables (Appendix-2). The software Statistica 7.0 was used for all these analysis.

4. Results and discussion

4.1. pH, temperature and nutrients in seawater

During the experiment, the temperature in the cylinders was kept around 24°C on average. Regarding the pH, the first ten days it was in a range of 7.8 and 8.8, and the last days of the experiment reached values of 9.0, indicating a high photosynthetic activity, without differences among treatments.

Phosphates were kept below the equipment detection limit.

In Table 4.1 nitrate and ammonium uptake rate efficiencies per day (%·day-^{1}) are shown calculated from measurements of the water samples for the three different nutrient concentrations under PAR and PAB treatment for different intervals of time: Day 3-7, day 6-7 and day 10-14.

Table 4.1 Uptake rate efficiencies per day (%·day-^{1}) of NO_3^- and NH_4^+ for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) for day 3 to 7 and for day 7 to 14.

		NO3- (%day^{-1})			NH4+ (%day^{-1})		
		Day 3 to7	Day 6 to7	Day 10 to 14	Day 3 to 7	Day 6 to 7	Day 10 to 14
PAR	60 µM	31.8	64.1	24.9	0.0	91.1	23.7
	480 µM	33.2	2.0	6.3	4.9	52.7	24.8
	900 µM	22.9	3.2	5.2	0.0	37.8	24.3
PAB	60 µM	32.3	91.1	25.0	1.8	67.3	24.6
	480 µM	33.3	11.9	5.3	0.0	20.9	24.4
	900 µM	25.6	2.4	3.6	0.0	99.2	23.6

For the lowest concentration (60 uM), the first three days they assimilate almost everything in nitrate. On days 6 to 7 and 10 to 14 they barely assimilate in the treatments with more nutrients On day 6 the water was changed and for this reason, this range of day 6 to 7 is taken and the theoretical value is taken as initial.

4.2. Growth rate

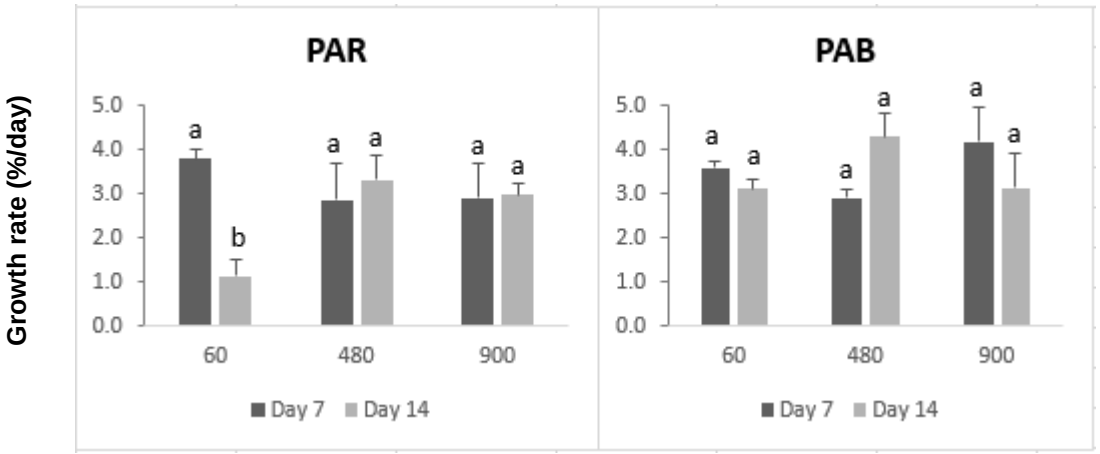

Figure 4.1. Growth rate for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) after 7 and 14 days. Data are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments.

Significant difference was found for the interaction of the three factors: nutrients, light and day. The smallest value was reached for 60µM under PAR treatment for 14 days of culture. However, no differences were found for the other treatments. These results suggest, in general, that *R. okamurae* is not affected to a great extent by the environmental factors, and is capable of maintaining a stable growth rate regardless of the light in the

culture and the nutrients introduced to the medium, confirming the enormous ability of this species to grow and spread through the invaded areas of the Strait.

4.3. Photosynthesis

The optimal quantum yield (Fv/Fm) was measured after 15 min (Fv/Fm_{15min}) and after 11.5 h (Fv/Fm_{night}) of darkness (at 8:30 a.m). Significant differences in the parameter Fv/Fm_{night} was observed for the interaction between day and nutrient and between light and nutrient (data not shown). For day and nutrient interaction, the Fv/Fm_{night} remained around 0.7 throughout the entire experiment and, independently of the days, lower Fv/Fm_{night} values were obtained at low nitrate concentrations. For light and nutrient interaction, significant differences were shown for PAB light treatment at 60µM, being Fv/Fm_{night} lower than in the rest of the treatments, so it is observed a negative UV effect. Otherwise, Fv/Fm_{15min} showed significant differences for the interaction between day and light (Table 4.2). At day 7 and 10 it was observed a negative effect of UV radiation in the parameter Fv/Fm_{15min}, however, no differences were found for the other days of experimentation.

It can be concluded that the UV and blue light at low concentration of nutrient have a negative effect on Fv/Fm_{night}. UV provokes photoinhibition but when high nitrogen level is high, the photoinhibition decreased. The positive effect of nitrate supply on photosynthetic activity and the decrease of photoinhibition have been observed in other algae both under laboratory conditions and under solar radiation (Barufi et al., 2012; Celis-Plá et al., 2015; Figueroa et al., 2021). Effective quantum yield was measured at 3 p.m. to assess the decrease related to increased irradiance as consequence of closure of reaction centers in algae under PAB treatment compared to PAR treatment.

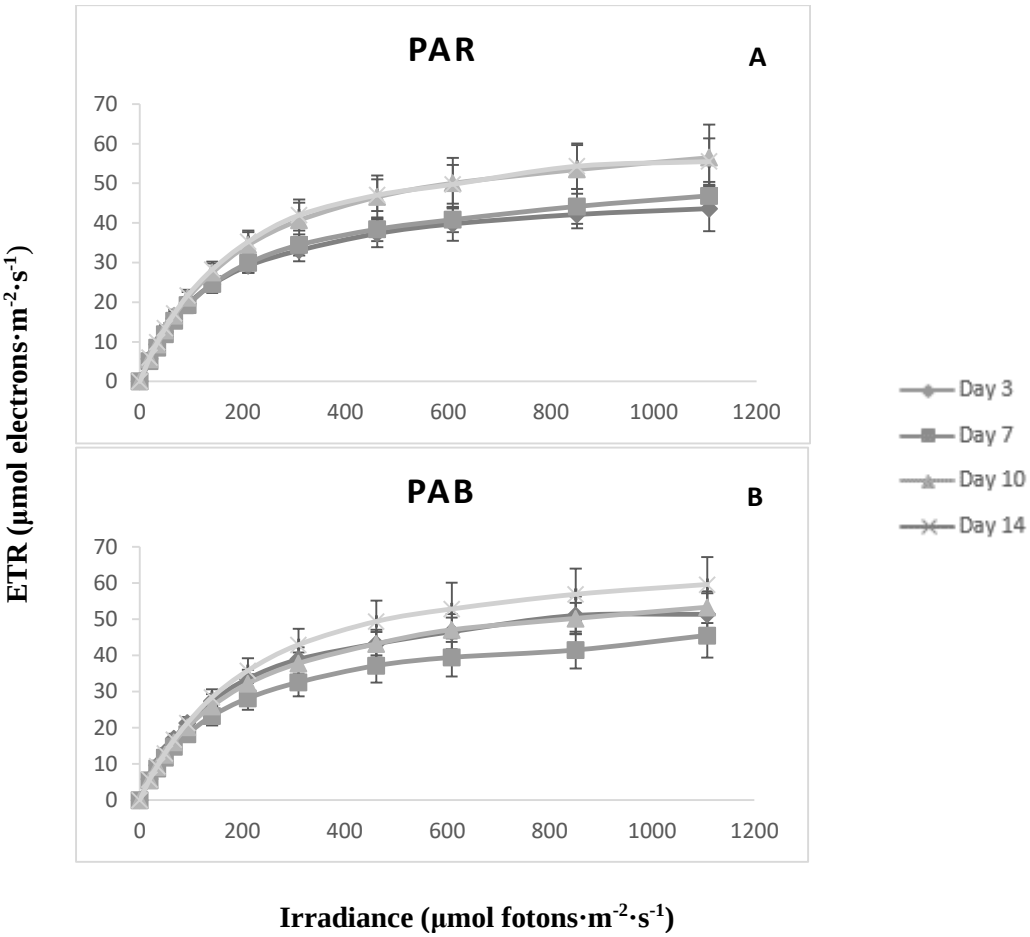

Figure 4.2. Rapid Light Curves (RLC) for *Rugulopteryx okamurae* exposed to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) under two light treatments: PAR (A) and PAB (B) after days 3, 7, 10 and 14 of experimentation. Data are pooled for factor nutrient and are expressed as mean + SD (n=3).

The results for $ETR_{in\ situ}$ measured at 3 p.m. showed that there were significant differences in this parameter for the interaction between the three factors (day, light and nutrient). The maximum $ETR_{in\ situ}$ value was reached on day 3 under PAB light at 900µM and the minimum on day 3 with PAB light at 60µM, so it can be concluded that ETR_{insitu} is positively affected by PAB light at high concentrations of nitrate. As to ETR_{insitu} compared to the ETR_{exsitu}, it was observed that the first presents higher levels than the second, as reported Figueroa et al (2021). If we look at the graph of the RLCs (Figure 4.2) on the x-axis, in the range from 300 to 330 (µmol photons·m^{-2}·s^{-1}), values between 30-45 µmol electrons·$m^{-}2$·s^{-1} are reached for ETR_{exsitu} both for the PAR and PAB treatment, while for the ETR_{exsitu} (calculated as yield·E_{PAR}·F_{II}·A) the range goes from 85-150 µmol·$m^{-}2$·s^{-1}.

Table 4.2. Maximal quantum yield (Fv/Fm_{15min}) and Rapid Light Curves (RLC) parameters (α_{ETR}, Ek_{ETR}, ETR_{max} and NPQ_{max}) and rate ETR_{max}/NPQ_{max} for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) during days 3, 7, 10 and 14. Data are expressed as mean + SD (n=3). Superscript letters denote significant differences among treatments.

			Fv/Fm_{15min}	α_{ETR}	Ek_{ETR}	ETR_{max}	NPQ_{max}	ETR_{max}/NPQ_{max}
Day 3	PAR	60 µM	0.730 ± 0.015^{ab}	0.283 ± 0.021	121.50 ± 24.00^{b}	33.88 ± 3.80^{b}	1.970 ± 0.569^{a}	18.05 ± 4.64^{e}
		480 µM	0.719 ± 0.015^{ab}	0.260 ± 0.044	157.19 ± 35.64^{b}	39.80 ± 4.13^{b}	1.260 ± 0.274^{bcd}	32.65 ± 7.84^{e}
		900 µM	0.721 ± 0.023^{ab}	0.250 ± 0.017	148.30 ± 28.23	36.78 ± 8.01^{b}	1.780 ± 0.243^{ab}	21.16 ± 6.66^{e}
	PAB	60 µM	0.719 ± 0.036^{ab}	0.277 ± 0.012	155.93 ± 12.54^{b}	43.43 ± 3.17^{b}	2.683 ± 1.306^{a}	18.19 ± 6.23^{e}
		480 µM	0.713 ± 0.031^{ab}	0.280 ± 0.050	140.12 ± 30.11^{b}	38.47 ± 3.21^{b}	1.983 ± 0.482	19.87 ± 2.98^{e}
		900 µM	0.723 ± 0.026^{ab}	0.253 ± 0.038	194.11 ± 29.16^{b}	48.40 ± 7.67^{b}	1.517 ± 0.421^{abc}	34.80 ± 16.06^{e}
Day 7	PAR	60 µM	0.740 ± 0.020^{a}	0.250 ± 0.020	164.52 ± 35.46^{b}	41.67 ± 11.20^{b}	1.027 ± 0.015^{cde}	40.59 ± 10.86^{de}
		480 µM	0.738 ± 0.016^{a}	0.297 ± 0.040	147.52 ± 20.19^{b}	43.67 ± 5.96^{b}	1.213 ± 0.280^{cde}	37.35 ± 9.94^{de}
		900 µM	0.725 ± 0.018^{a}	0.240 ± 0.010	181.63 ± 17.95^{b}	43.74 ± 5.42^{b}	1.297 ± 0.388^{cd}	35.74 ± 11.60^{de}
	PAB	60 µM	0.693 ± 0.062^{bc}	0.303 ± 0.035	146.15 ± 24.34^{b}	43.86 ± 1.78^{b}	0.783 ± 0.049^{de}	56.16 ± 4.61^{c}
		480 µM	0.679 ± 0.070^{bc}	0.270 ± 0.046	174.76 ± 28.91^{b}	46.32 ± 1.89^{b}	0.937 ± 0.051^{cde}	49.63 ± 4.84^{c}
		900 µM	0.679 ± 0.060^{bc}	0.267 ± 0.038	173.46 ± 23.95^{b}	45.41 ± 4.60^{b}	0.857 ± 0.112^{de}	53.27 ± 4.85^{c}
Day 10	PAR	60 µM	0.722 ± 0.023^{ab}	0.293 ± 0.031	191.02 ± 31.45^{a}	55.34 ± 9.81^{a}	1.273 ± 0.032^{cd}	43.36 ± 6.61^{cd}
		480 µM	0.710 ± 0.013^{ab}	0.280 ± 0.030	181.30 ± 12.01^{a}	50.55 ± 4.09^{a}	1.100 ± 0.229^{cde}	47.36 ± 10.50^{cd}
		900 µM	0.681 ± 0.028^{ab}	0.267 ± 0.025	198.05 ± 20.12^{a}	53.04 ± 6.61^{a}	0.943 ± 0.072^{cde}	56.40 ± 7.47^{cd}
	PAB	60 µM	0.633 ± 0.040^{c}	0.283 ± 0.021	206.53 ± 36.86^{a}	58.97 ± 13.94^{a}	0.560 ± 0.135^{e}	106.8 ± 22.60^{a}
		480 µM	0.685 ± 0.017^{c}	0.290 ± 0.020	174.27 ± 20.38^{a}	50.52 ± 8.78^{a}	0.707 ± 0.107^{de}	73.23 ± 20.14^{a}
		900 µM	0.676 ± 0.017^{c}	0.313 ± 0.015	184.04 ± 47.64^{a}	56.90 ± 12.78^{a}	0.673 ± 0.071^{de}	84.13 ± 12.80^{a}
Day 14	PAR	60 µM	0.695 ± 0.011^{abc}	0.297 ± 0.021	184.77 ± 22.91^{a}	54.82 ± 3.82^{a}	0.853 ± 0.064^{de}	64.71 ± 9.44^{b}
		480 µM	0.700 ± 0.035^{abc}	0.290 ± 0.030	196.24 ± 30.32^{a}	57.04 ± 14.56^{a}	0.810 ± 0.082^{de}	72.12 ± 26.34^{b}
		900 µM	0.704 ± 0.022^{abc}	0.283 ± 0.023	203.55 ± 19.67^{a}	57.49 ± 8.40^{a}	0.710 ± 0.053$_{de}$	80.79 ± 7.90^{b}
	PAB	60 µM	0.730 ± 0.005^{a}	0.303 ± 0.015	204.49 ± 25.10^{a}	62.43 ± 7.74^{a}	0.850 ± 0.213^{de}	77.45 ± 26.49^{b}
		480 µM	0.743 ± 0.021^{a}	0.330 ± 0.010	172.38 ± 15.03^{a}	56.49 ± 4.06^{a}	0.770 ± 0.110^{de}	73.87 ± 5.44^{b}
		900 µM	0.731 ± 0.007^{a}	0.323 ± 0.032	150.27 ± 17.22^{a}	47.99 ± 2.43^{a}	0.693 ± 0.081^{de}	69.74 ± 7.80^{b}

Note: For α_{ETR}, significant differences were found for day and light factors independently, without interaction between factors. Statistical results on the text.

The adjustment of the Rapid Light Curves (RLC) to obtain the photosynthetic parameters α_{ETR}, Ek_{ETR}, ETR_{max} and NPQ_{max} was done with the R program.

Significant differences were found for day and light factors independently, without interaction between factors for the photosynthetic efficiency (α_{ETR}). It was significantly lower after 3 and 7 days than after 14 days of culture. For factor light, α_{ETR} was significantly lower under PAR than under PAB, thus a positive effect of UV radiation on this parameter was observed.

Significant differences were found for the factor "day" for the light saturation irradiance (Ek_{ETR}). The highest values were observed for days 7 and 14 of the experiment. The photosynthetic parameter ETR_{max} was also affected by the "day" factor, being higher the same days as for Ek_{ETR}.

For ETR_{max}/NPQ_{max}, significant differences were reported for the interaction between light and "day". ETR_{max}/NPQ_{max} is a good indicator of production with respect to energy dissipation. This proportion is better related to each one separately (Figueroa et al., 2014). The highest value of this rate were reached on day 14 and the lowest on day 7, for the two light treatment respectively.

Finally, UVR at these doses studied in *R. okamurae*, would act as an inducing mechanism for the biosynthesis of photoprotective and antioxidant compounds, allowing this species to achieve a homeostatic balance against the stress generated by exposure to UVR (Rastogi et al. 2017).

4.4. Elemental analysis (internal C, N, S)

For internal C content it was found a significant interaction between light and nutrient. Higher amount of C ($mg \cdot g^{-1}$ DW) was reached for 900 and 60µM under PAR treatment (Figure 4.3).

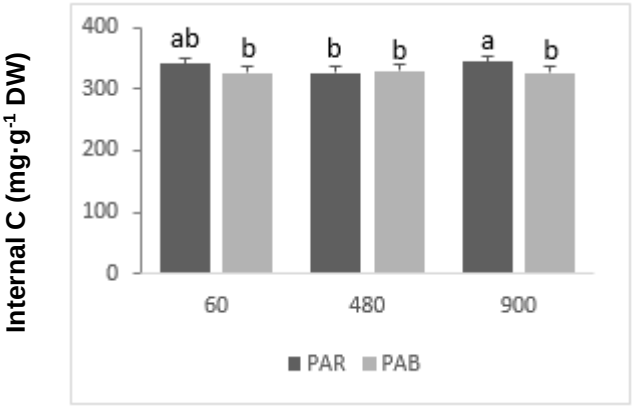

Figure 4.3. Internal C expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are pooled for factor day and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value = 304.7 mg · g^{-1} DW.

Significant differences were found for internal N content for factors nutrient, light and "day" independently, without showing interaction among factors. Internal N content was higher for the nutrient concentration 480 and 900µM, as can be seen in Figure 4.3 A. Regarding the light treatment, it is higher in PAR than in PAB (Figure 4.3 B) and increased over time, with a higher content at 14 days (Figure 4.3 C).

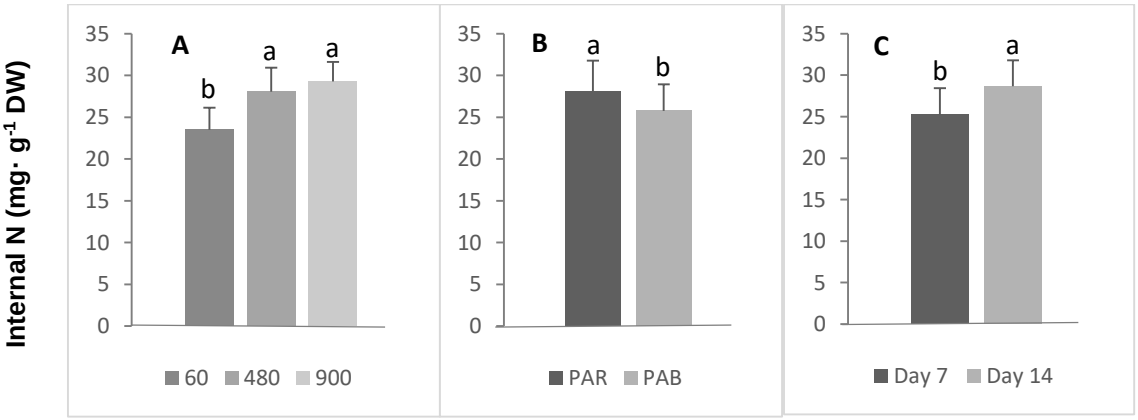

Figure 4.4. Internal N expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) for 7 and 14 days. Data are pooled for factor light and day (A), nutrient and day (B) and nutrient and light (C) and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 15.6 mg· g^{-1} DW.

Significance difference were found for C/N for the interaction between day and light (Figure 4.4 A) and for nitrate independently (Figure 4.4 B). For the first case, higher internal C/N values were reported on day 7 under PAB light and regarding to the nutrient factor, there was a greater amount of C/N for the smallest concentration (60µM).

Nitrate reductase activity (NRA) is closely related to nitrogen incorporation rates in macroalgae (Davison et al., 1984) and is known to be stimulated by NO_3^- in cultured algae (Gao et al., 1995; Lartigue et al., 2005). NRA is regulated by light with rapid suppression of NRA in darkness in most algae studied (Vergara et al., 1998; Lartigue et al., 2002). In addition, NRA in algae is photoregulated by Blue/UVA photoreceptor (López-Figueroa et al., 1991). The fact that the enzyme is regulated by light implies that perhaps in a treatment with blue light and UVA, like the PAB in this study, it makes the enzyme accelerate and favors the incorporation of N in comparison with the PAR treatment.

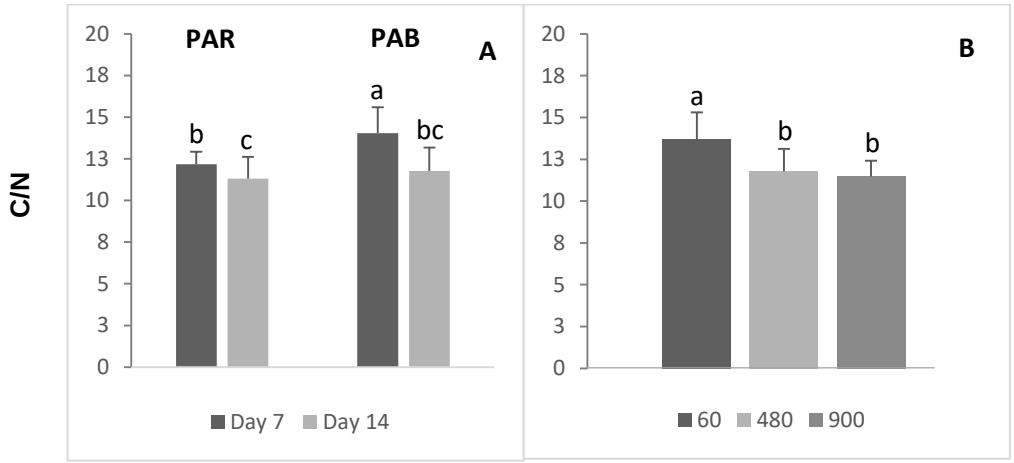

Figure 4.5. Internal C expressed as $mg \cdot g^{-1}$ DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are pooled for factor nutrient (A) and for light and day (B) and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 19.5 $mg \cdot g^{-1}$ DW.

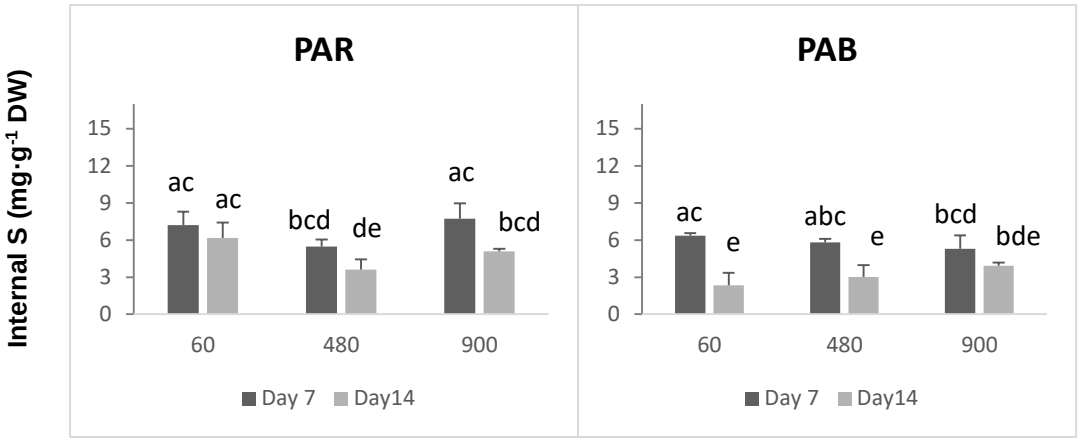

Figure 4.6. Internal S expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 15.6 mg· g^{-1} DW.

For internal S, significant difference for the interaction between light, day and nitrate were reported. Higher values of internal S were reached for 900µM under PAR and for 60µM under PAB on day 7 and just the opposite occurred on day 14.

4.5. Photosynthetic pigments

In general, pigments were not affected by light. Chlorophyll *a* (Chl*a*) was affected only by factor nutrient, without interaction between factors. The concentration of Chl*a* increases with the concentration of N, despite the fact that it is a non-nitrogenous compound.

Chl(*c1+c2*) and carotenoids levels were affected by day and nutrient as independent factors. These pigments also increase with nitrate concentration, such as Chl*a*, and in addition, Chl(*c1+c2*) increases after 14 days and the opposite for carotenoids

These results suggest that, a high availability of nutrients under favourable conditions of quantity and light quality, as a photoregulatory process, simulate the accumulation of photosynthetic pigments in algae *R. okamurae* (Rüdiger et al., 1992).

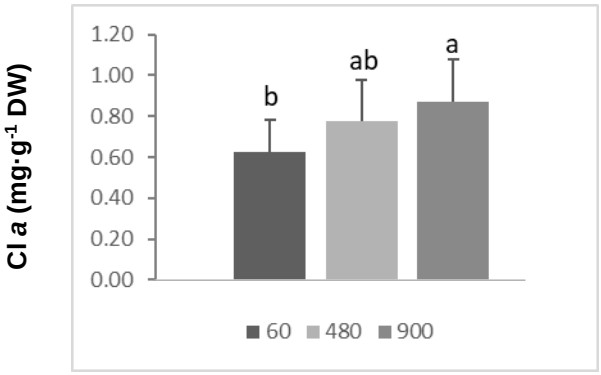

Figure 4.7. Chlorophyll *a* expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are pooled for day and light and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 0.53 mg· g^{-1} DW.

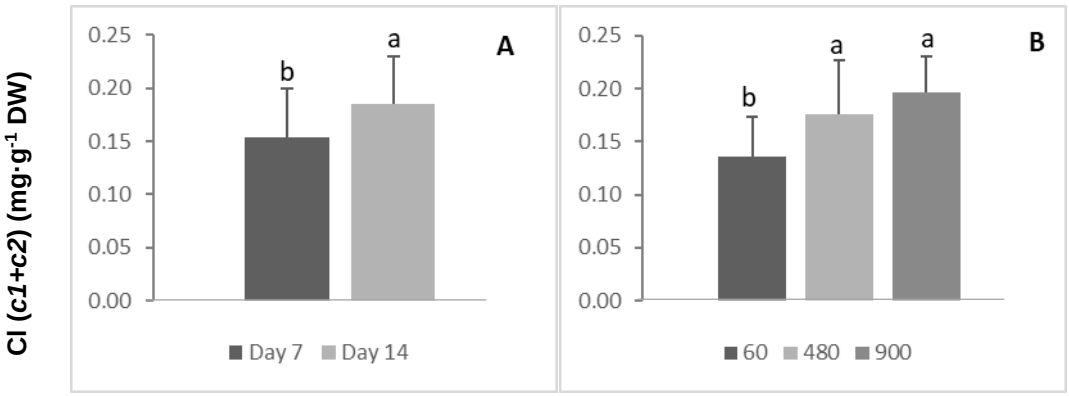

Figure 4.8. Chlorophyll (*c1+c2*) expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are pooled for light and nutrient (A) and for day and light (B) and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 0.10 mg· g^{-1} DW.

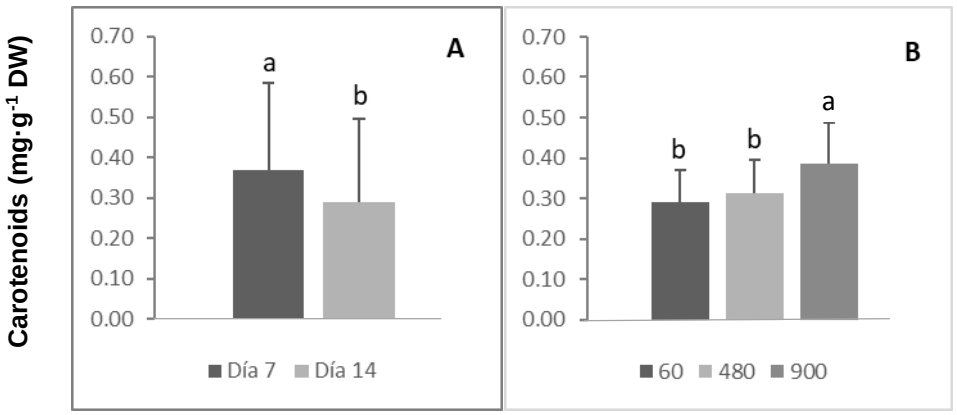

Figure 4.9. Carotenoids expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate). Data are pooled for light and nutrient (A) and for day and light (B) and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 0.25 mg· g^{-1} DW.

A positive influence of N has been observed in all pigments. It is curious that the light has not affected them, when it would have been expected, this may be because the amount of UV and the quality, being UVA, has not affected negatively.

4.6. Total carbohydrates, soluble protein and total lipids

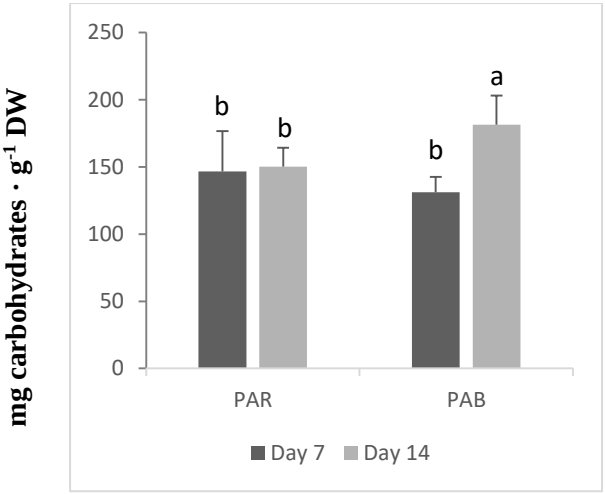

Figure 4.10. Total carbohydrates content for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) after 14 days. Data are pooled for factor nutrient and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value = 138.4 mg · g^{-1} DW.

Carbohydrates were affected by the interaction between day and light. Significant differences were found for PAB light on day 14 and in this treatment the highest value of carbohydrates was obtained. The amount of carbs increased compared to the initial value. Therefore, it can be affirmed that PAB light treatment had a positive effect on carbohydrate synthesis after 14 days.

In general terms, carbohydrate content increases with UV and blue light supplementation (Pagels et al., 2020). Considerable amount of carbohydrates was reported for *R. okamurae*

in the present work, as lower values of carbohydrate content was reported in other brown algae *Ascophyllum nodosum* (Różyło et al., 2017), *Cystoseira compressa*, *Dictyota dichotoma*, *Padina pavonia*, *Sargassum vulgare*, *Stypopodium schimperi*, *Cystoseira corniculata*, *Cystoseira barbata* and *Stypocaulon scoparium* (Ozgun et al., 2015), *Padina tetrastromatica* (Jose et al., 2015) and *Hydroclathrus clathratus* (Awad et al., 2009).

Soluble protein

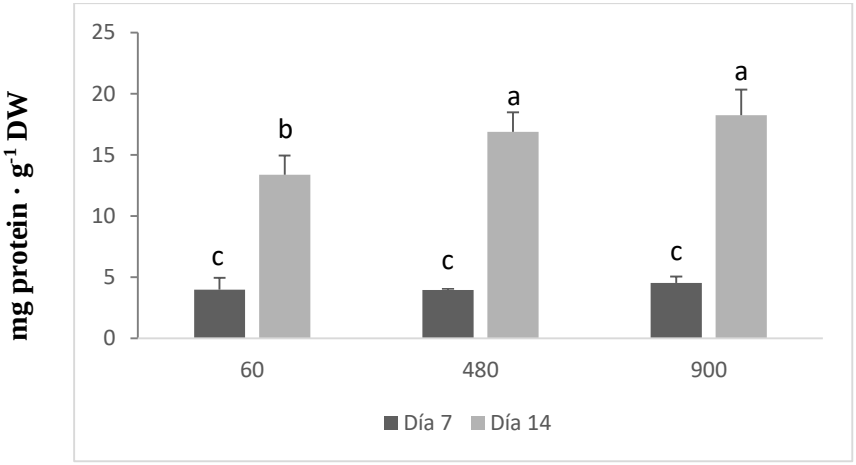

Figure 4.11. Soluble proteins expressed as mg·g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) after 7 and 14 days. Data are pooled for light factor and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value= 3.01 mg · g^{-1} DW.

The concentration of soluble proteins increases with "day" factor, being higher on day 14 than on 7 for each concentration nutrient. It can be seen that the amount of protein increases over time and with a higher nitrogen content. Proteins are made up of nitrogen (N), which is vital for their synthesis of DNA and RNA (Richmond, 2004). The values of protein content obtained in the present work, which are on average around 4 mg · g^{-1} DW for day 7 and around 15 mg · g^{-1} DW for day 14, are similar to other observed in *Ecklonia maxima*, *Laminaria pallida* and *Splachnidium rugosum* (January et al., 2019). The protein content of marine algae changes a lot with species, seasons and nutrients (Stengel et al., 2011). A reduction of soluble proteins was observed in different algae, such as Rubisco under low nitrogen conditions (Beardall et al., 1991).

The accumulation of C and N compounds as photoregulation of metabolism for the production of bioactive compounds is affected by the quality of light, such that nitrogen

compounds are favoured by blue light (although this has not been observed because in the case of proteins there were no significant differences between the light treatments) and carbon compounds by red light (Torres et al., 1995); Aguilera et al., 2000).

Total lipids

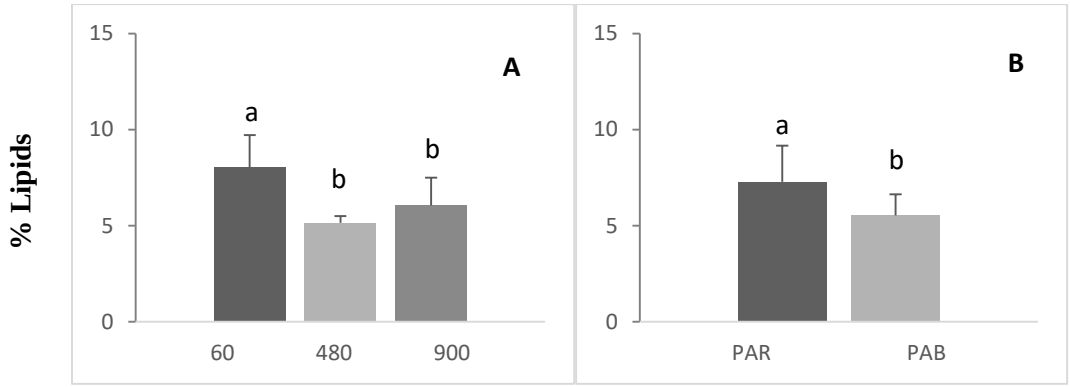

Figure 4.12. Percentage of lipids for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) after 14 days. Initial value was indicated as lined bar. Data are pooled for factor light and day (A), nutrient and day (B) and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value = 11%.

In general, at low concentrations of nitrate there is a higher lipid content (Figure 4.12 A) and with UV light it decreases (Figure 4.12 B). The total lipids content (%) decreased for the two factors (nutrients and light) with respect to the initial samples. A low availability of N in the culture medium stimulate the synthesis and accumulation of lipids (Griffiths et al., 2009). As we found, it has been reported that the microalgae *Nannochloropsis oculata* and *Chlorella vulgaris* increases total lipid under nitrogen limitation. However, *Phaeodactylum tricornutum* increased EPA and PUFA after UV radiation induction (Sharma et al., 2012) and in this work it was observed a decrease of total lipid content under UV radiation.

4.7. Phenolic compounds

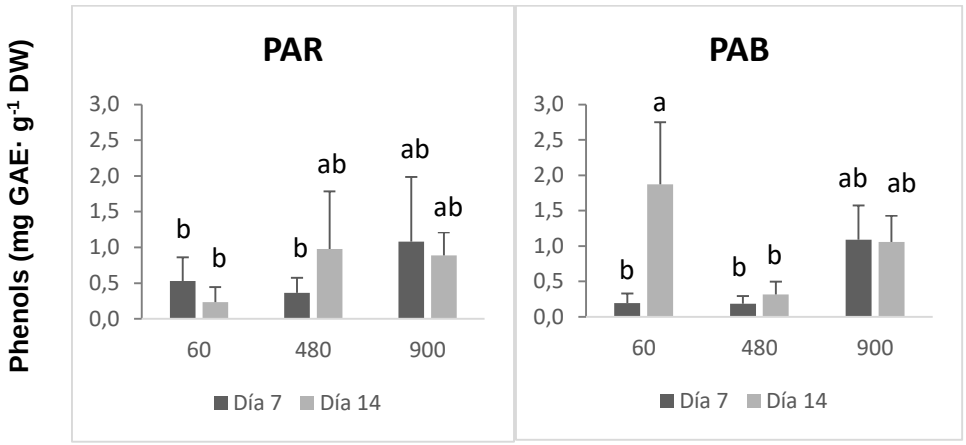

Figure 4.13. Phenols compounds expressed as mg gallic acid equivalent (GAE)· g^{-1} DW for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) after 7 and 14 days. Data are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value = 0.94 mg GAE· g^{-1} DW.

Phenolic content was affected by the interaction of light, day and nitrate. The only treatment that presented a significant difference compared to the others was on day 14 for PAB at 60 µM, where highest levels of phenols were reached. In general terms, no clear trend is observed in the effect of nutrients and light on the concentration of phenols.

If the average of days 7 and 14 is taken, values of 0.57 and 0.89 mg GAE g^{-1} DW were obtained. Similar concentrations have been described in the other brown macroalgae, *Sargassum cymosum*, *Sargassum sp*. and *Dictyota sp*. (Echavarría et al. 2009), and higher in *Padina australis* (Thiyagarasaiyar et al., 2021). The GAE amount mentioned above is low compared to the value of 6 mg phloroglucinol·g^{-1}DW on average obtained for *Rugulopteryx okamurae* in other work (Huesa-Cerdán et al. 2020) because PVPP was used in the present work to precipitate proteins which could interfere with phenolic compounds. Without using PVPP, an average of 2.50 mg GAE·g^{-1}DW was obtained. Similar values were obtained in *Macrocystis pyrifera* (Castro et al. 2016).

Phenolic compounds are involved in mechanisms of photoprotection against solar radiation, especially UV radiation (Pavia et al. 1997). When seaweeds are exposed to high levels of PAR or UVR, they must protect their photosynthetic apparatus either by automatic shading or by synthesizing photoprotective compounds such as mycosporine-like amino acids in red macrolagae (Korbee et al. 2006) and phenolic compounds in

brown macroalgae. The increase in the availability of N under favourable conditions of quantity and light quality, as a photoregulatory process, favors the accumulation of non-nitrogenous compounds such as phenolic compounds (Celis-Plá et al. 2016).

4.8. Antioxidant capacity

No significant differences were found in the statistical results among treatments, but the alga has a very high antioxidant capacity since for the antioxidant activity in percentage (AA%) values of 98% were obtained on average. There were no differences between the initial value and the values obtained after the experiment.

Table 4.3 Antioxidant activity (ABTS and DPPH) for *Rugulopteryx okamurae* initially and exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60 µM, 480 µM and 900 µM of nitrate) for 7 and 14 days. Data are expressed as mean + SD (n=3). The value was expressed as µmol of Trolox equivalent per gram of dry weight (µmol TE·g^{-1}DW). Superscript letters denote significant differences among treatments. For DPPH, A: day 7, B: day 14, a: PAR and b: PAB.

		ABTS		DPPH	
Initial		21.09±	0.34	10.79±	1.74
Day 7	PAR 60 µM	20.75±	0.67^{a}	9.38±	0.97^{Aa}
	PAR 480µM	21.00±	0.16^{a}	9.29±	0.41^{Aa}
	PAR 900µM	20.99±	0.09^{a}	9.23±	1.92^{Aa}
	PAB 60 µM	21.08±	0.43^{a}	7.82±	2.40^{Ab}
	PAB 480µM	20.93±	0.31^{a}	8.57±	1.74^{Ab}
	PAB 900µM	21.00±	0.14^{a}	6.91±	1.68^{Ab}
Day 14	PAR 60 µM	21.29±	0.22^{a}	6.64±	2.43^{Ba}
	PAR 480µM	21.48±	0.21^{a}	6.89±	2.90^{Ba}
	PAR 900µM	20.95±	0.43^{a}	7.33±	2.23^{Ba}
	PAB 60 µM	21.15±	0.22^{a}	5.69±	0.68^{Bb}
	PAB 480µM	20.78±	0.12^{a}	5.40±	0.85^{Bb}
	PAB 900µM	21.03±	0.31^{a}	5.43±	0.95^{Bb}

It can be said that *R. okamurae* has a high antioxidant capacity obtained by ABTS method. ABTS assay carried out for *A. nodosum* and *Schizochytrium* spp. in ethanol extract exhibited an antioxidant activity of 0.75 ± 0.31 µmol TE·g^{-1}DW and 2.56 ± 0.53 µmol TE/g^{-1}DW, respectively (Dell'Anno et al. 2020).

Significant differences were found for DPPH for factors day and light independently. On the one hand, TE values reached on day 7 were higher than on day 14 (Table 4.3).

Moreover, treatment with PAR light had a positive effect in terms of DPPH (µmol TE·g^{-1}DW) compared to PAB treatment. It can be said that during the first week of the experiment with PAR light, the algae had more antioxidant capacity. Antioxidant capacity measured by the ABTS method is greater than that measured by the DPPH method, as reported by Vega et al. (2020) for the case of cyanobacteria and species of red algae.

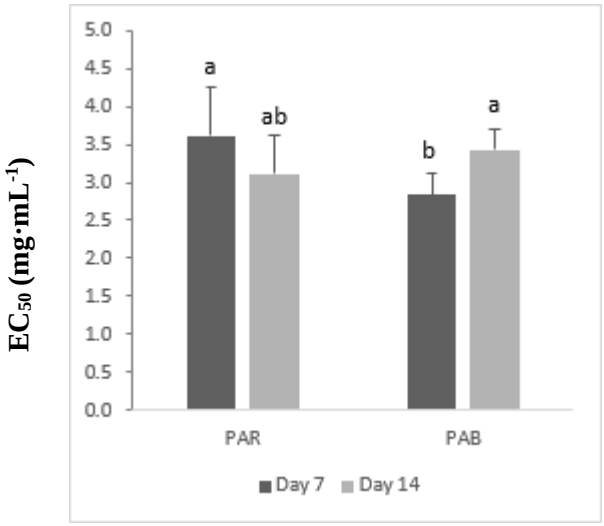

Figure 4.14. EC_{50} (mg·mL^{-1}) obtained by DPPH method for *Rugulopteryx okamurae* exposed to two light treatments (PAR and PAB) and to three different nutrient concentrations (60µM, 480µM and 900µM of nitrate) during days 7 and 14. Data are pololed for nutrient factor and are expressed as mean + SD (n=3). Lowercase letters denote significant differences among treatments. Initial value of EC_{50} (mg·mL^{-1}) = 3.05

Statistical analyses for EC_{50} (mg·mL$^{-1)}$ show that there were significant differences in the interaction between day and light. EC_{50} is the amount of alga necessary to reduce the amount of DPPH added by half, so the lower the EC_{50}, the higher the antioxidant capacity. A higher antioxidant activity was shown by the samples on day 7 with PAR light treatment (3.62 ± 0.64 mg·mL^{-1}). On day 14, PAR light has a positive effect compared to the PAR light.

Similar values (0.005 and 0.017 mg·mL^{-1} DW) of EC_{50} (oxidation index) were obtain for brown macroalgae *Cystoseira tamariscifolia* and *Padina pavonica* (Korbee et al., 2014). Antioxidant activity of brown alga *Saccharina bongardiana* obtained by DPPH method in methanol extract reported a result of EC_{50} of 0.023 µg DW·mL^{-1} (Auezova et al., 2013), for other brown algae, *Fucus vesiculosus* in 80% methanol extract, 7.54 ± 0.36 (µg·mL^{-1}) and radical scavenging activity of different solvent fractions showed the

following results for EC_{50} ($\mu g \cdot mL^{-1}$): 3.76 ± 0.22 with ethyl acetate and 4.77 ± 0.25 with 1- butanol solvents respectively (Wang et al., 2012). The results mentioned above translate into a lower antioxidant capacity of the algae studied compared to that obtained in this work. Similar values were obtained for brown algae *Fucus spiralis Linnaeus* and *Treptacantha abies-marina* (Vega et al., 2020).

4.9. Assessment of the photoprotective capacity of cosmetic preparations (SPF)

No significant differences were found after statistical analyses for SPF for pure extract (aqueous and hydroalcoholic at 10 and 15%, respectively) and cosmetic cream prepared with the algal extract.

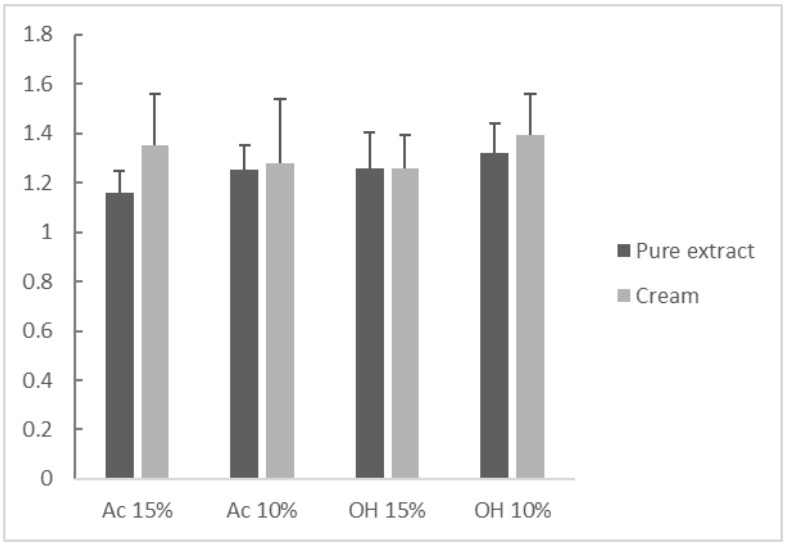

Figure 4.15. SPF for pure extract and cream prepared in the two solvents: aqueous (Ac) and hydroalcoholic (OH) at two different concentrations (10 and 15%). Data are expressed as mean + SD (n=3). Control cream (SPF)= 1.19.

The application of algae extracts in combination with other natural ingredients, can help design a new generation of photoprotectors against the negative effects of UVR and photoaging produced by blue light and UVA. In addition, it has enormous advantages related to its high photostability and thermostability for not presenting toxicity to both human health and the environment since it is a biodegradable product. Finally, at the topical level, the formulation provides great hydration for the skin due to the synergy of the seaweed extract with natural substances.

5. Relation between variables

A positive correlation was observed between internal C ($mg \cdot g^{-1}$ DW) and α_{ETR} (r = -0.42, n =36; p <0.05). No correlations were found for growth, internal C and N ($mg \cdot g^{-1}$ DW) and photosynthetic parameters.

Proteins were correlated with α_{ETR} (r = 0.44, n =36; p <0.05) and ETR_{max}/NPQ_{max} (r = 0.71, n =36; p <0.05) and the same for carbohydrates (r = 0.35, n =36; p <0.05) and (r = 0.38, n =36; p <0.05), respectively. For lipids was found a positive correlation with Fv/Fm_{15min} (r = 0.42, n =36; p <0.05) and a negative correlation with ETR_{max}/NPQ_{max} (r = -0.49, n =36; p <0.05). These results suggest that, UVR could be a very useful mechanism for induction or stimulation of secondary metabolism in algae. The limitation of N in the aquatic environment increases the sensitivity to UVR and causes a decrease in photosynthesis (Litchman et al. 2002).

Antioxidant capacity measured by ABTS method was positively correlated with Chl(*c1+c2*) (r = 0.35, n =36; p <0.05) and negative correlated with ETR_{insitu} (r = -0.37, n =36; p <0.05).

On the other hand, antioxidant capacity measured by DPPH assay in terms of µmol $TE \cdot g^{-1}$ DW, present a positive correlation with Fv/Fm_{15min} (r = 0.40, n =36; p <0.05) and a negative correlation with α_{ETR} (r = -0.44, n =36; p <0.05) and ETR_{max}/NPQ_{max} (r = -0.40, n =36; p <0.05). For EC_{50} ($mg \cdot mL^{-1}$) significant correlations were found for Fv/Fm_{night} (r = 0.35, n =36; p <0.05).

Regarding to the bioactive compounds, antioxidant capacity was positively correlated with lipids (r = 0.35, n =36; p <0.05) and negatively correlated with proteins (r = -0.50, n =36; p <0.05) for µmol $TE \cdot g^{-1}$ DW (DPPH method). No correlations were found for ABTS and EC_{50} in the production of bioactive compounds and the antioxidant activity of any method was not correlated with phenols.

6. Conclusions

In general terms, *Rugulopteryx okamurae* is a species capable of proliferating independently of UV light and different concentrations of nutrients, although UV

radiation is more influential on the physiology of this species. This suggests that the species has great adaptability, thanks to which it has been able to invade and occupy the waters of the Strait and its surroundings, benefiting from the gradual increase in global warming, due in part to UV radiation, it is very likely that this species continues to spread along the Andalusian coasts, even far from areas rich in nutrients such as the Gulf of Cadiz.

- Data have been obtained on all the photosynthetic parameters of *R. okamurae*, as well as how they differ according to the environmental factors studied. No interactive effects were found for growth and photosynthesis, but there were for the ability to produce bioactive compounds such as proteins, lipids and carbohydrates with photosynthetic activity.

- The maximum quantum yield (FSII) of photosystem II was affected by ultraviolet light, the maximum electron transport capacity (ETR_{max}) and the saturation irradiance (E_k) were only affected by the "day" factor. The αETR was not affected by light or nitrates, nor by their interaction. Finally, the ETR_{max} / NPQ_{max} ratio was affected by the interaction between nutrient concentration and the "day" factor.

- With the exception of the antioxidant capacity measured by the ABTS test, all the biomolecules analyzed changed depending on the environmental factors studied, highlighting an increase in the synthesis of these biomolecules with the effect of UV light in lipids and carbohydrates. The availability of nutrients, on the other hand, also plays a fundamental role in the synthesis of these biomolecules. Proteins were strongly affected by the latter, increasing its concentration.

- The cosmetic preparations prepared from the extracts have not been shown to have a great capacity for protection, although *R. okamurae* does have the potential to be used in cosmeceutical due to its great antioxidant capacity.

In short, it has been possible to successfully design a multifactorial experiment that has provided fundamental information on the physiology of an invasive macroalgae species of great importance, and in which the viability of its cultivation in laboratory conditions has been demonstrated, laying the foundations for new research and solutions against this threat to coastal ecosystems.

7. Future research lines

In view of these results, it is still necessary to study other physiological aspects of *Rugulopteryx okamurae*, among others its capacity for reproduction under multiple stress conditions and also the possible changes in its physiology as a function of other environmental factors such as salinity in the Water. Furthermore, the possibility of preserving *R. okamurae* under artificial conditions opens the doors to future experimental lines that study the persistence of this species and its development over a longer period of time.

Ocean acidification and temperature are other aspects to investigate. Given that this phenomenon increases the concentration of CO_2 available for photosynthesis, and it has been proven how this benefits brown macroalgae such as *Cystoseira tamariscifolia* (Celis-Plá et al., 2017), study and design new models that contribute to understanding the scope of expansion in the face of this consequence of climate change is essential. Also integrating all the data obtained from this experiment, a deeper characterization of *R. okamurae* could be achieved.

Regarding the levels of carbohydrates obtained, a strategy arises to promote the withdrawal of *R. okamurae* from the affected coasts, based on encouraging the economic interest of the exploitation of these algae. Fucoidan is a polysaccharide present in the cell walls of brown algae, and it is a molecule of great biotechnological interest due to its activity as an anticoagulant, anti-inflammatory or antioxidant (Dore et al., 2013). Several studies show that this compound promotes lipolysis in adipocytes (Park et al., 2011), and also apoptosis of colon cancer cells (Kim et al., 2010).

If the extraction of the fucoidan present in *R. okamurae* turns out to be economically viable, the creation of initiatives and projects for the safe extraction of this alga in the areas invaded by this species could be stimulated, for its processing and adaptation to tools for pharmaceutical use and / or nutraceuticals.

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Appendix 1– Statistycal analysis

D= days, N= nutrients, L= light, C= cylindrical vessel

Table S1: Statistical Test ANOVA for photosynthetic parameters

Variable	Source of variation	d.g.	M.S.	F	p
Fv/Fmnight	***C(L*N)***	***12***	***0.0004***	***2.5***	***0.005273***
	D	***3***	***0.0013***	***7.3***	***0.000118***
	L	***1***	***0.0021***	***12.2***	***0.000599***
	N	***2***	***0.0035***	***19.9***	***0.000000***
	D*L	3	0.0001	0.7	0.573437
	D*N	***6***	***0.0008***	***4.7***	***0.000169***
	L*N	***2***	***0.0017***	***9.9***	***0.000082***
	D*L*N	6	0.0002	0.9	0.491499
	Error	180	0.0002		

Variable	Source of variation	Effect	Degr. of	MS	Den.Syn.	Den.Syn.	F	p
ETR_{insitu}	***D***	***Fixed***	***3***	***344***	***180.0000***	***68.5115***	***5.02***	***0.002289***
	L	***Fixed***	***1***	***2013***	***12.0000***	***147.1318***	***13.68***	***0.003044***
	N	Fixed	2	555	12.0000	147.1318	3.77	0.053566
	D*L	Fixed	3	157	180.0000	68.5115	2.29	0.079666
	D*N	***Fixed***	***6***	***537***	***180.0000***	***68.5115***	***7.85***	***0.000000***
	L*N	Fixed	2	183	12.0000	147.1318	1.24	0.323034
	C(L*N)	***Random***	***12***	***147***	***180.0000***	***68.5115***	***2.15***	***0.016093***
	D*L*N	***Fixed***	***6***	***325***	***180.0000***	***68.5115***	***4.75***	***0.000162***
	Error		180	69				

Table S2: Statistical Test ANOVA for Rapid Light Curves (RLC)

Variable	Source of variation	d.g.	M.S.	F	p
Fv/Fm_{night}	***D***	***3***	***0.00490***	***5.08***	***0.003886***
	N	***1***	***0.00414***	***4.29***	***0.043705***
	L	2	0.00020	0.21	0.809716
	D*N	3	0.00667	6.91	0.000581
	D*L	***6***	***0.00038***	***0.40***	***0.878316***
	N*L	2	0.00081	0.84	0.436219
	D*N*L	6	0.00076	0.79	0.580370
	Error	48	0.00096		
αETR	***D***	***3***	***0.005235***	***6.169***	***0.001241***
	N	***1***	***0.005168***	***6.090***	***0.017211***
	L	2	0.001172	1.381	0.261048
	D*N	3	0.000412	0.486	0.693543

	D*L	6	0.000583	0.687	0.660676
	N*L	2	0.000672	0.792	0.458703
	D*N*L	6	0.001294	1.525	0.190184
	Error	48	0.000849		
Ek	***D***	***3***	***5330***	***7.476***	***0.000333***
	N	1	0	0.000	0.990331
	L	2	776	1.089	0.344759
	D*N	3	1220	1.711	0.177304
	D*L	6	945	1.325	0.264588
	N*L	2	739	1.036	0.362705
	D*N*L	6	1395	1.957	0.090665
	Error	48	713		
ETRmax	***D***	***3***	***1073.3***	***18.883***	***0.000000***
	N	1	123.1	2.166	0.147662
	L	2	12.7	0.223	0.800979
	D*N	3	41.9	0.738	0.534649
	D*L	6	48.7	0.857	0.533299
	N*L	2	48.6	0.855	0.431745
	D*N*L	6	47.2	0.830	0.552659
	Error	48	56.8		
NPQmax	***D***	***3***	***3.40758***	***63.472***	***0.000000***
	N	1	0.48840	9.097	0.004085
	L	***2***	***0.05732***	***1.068***	***0.351838***
	D*N	3	0.33667	6.271	0.001116
	D*L	***6***	***0.08521***	***1.587***	***0.171422***
	N*L	2	0.12710	2.367	0.104573
	D*N*L	***6***	***0.12336***	***2.298***	***0.049713***
	Error	48	0.05369		

Table S3: Statistical Test ANOVA for growth rate

Variable	Source of variation	d.g.	M.S.	F	p
Growth rate	***D***	***1***	***1.3340***	***4.548***	***0.043382***
	N	2	0.6981	2.380	0.114041
	L	***1***	***4.5647***	***15.563***	***0.000605***
	D*N	***2***	***4.6854***	***15.975***	***0.000039***
	D*L	1	1.0129	3.453	0.075427
	N*L	2	0.1054	0.360	0.701717
	D*N*L	***2***	***2.1026***	***7.169***	***0.003622***
	Error	24	0.2933		

Table S4: Statistical Test ANOVA for elemental analysis (internal C, N, S)

Variable	Source of variation	d.g.	M.S.	F	p
C (mg·g-1 DW)	***D***	***1***	***792***	***9.13***	***0.005891***
	N	2	193	2.23	0.129259
	L	***1***	***881***	***10.16***	***0.003957***
	D*N	2	23	0.26	0.769854
	D*L	1	47	0.54	0.468242
	N*L	***2***	***368***	***4.24***	***0.026502***
	D*N*L	2	14	0.17	0.847220
	Error	24	87		
N (mg·g-1 DW)	***D***	***1***	***106.67***	***47.80***	***0.000000***
	N	***2***	***110.96***	***49.71***	***0.000000***
	L	***1***	***51.62***	***23.13***	***0.000067***
	D*N	2	3.61	1.62	0.219660
	D*L	1	0.46	0.21	0.652290
	N*L	2	0.74	0.33	0.721224
	D*N*L	2	2.88	1.29	0.293659
	Error	24	2.23		
C/N	***D***	***1***	***22.174***	***43.24***	***0.000001***
	N	***2***	***17.332***	***33.80***	***0.000000***
	L	***1***	***12.069***	***23.54***	***0.000061***
	D*N	2	1.065	2.08	0.147379
	D*L	***1***	***4.470***	***8.72***	***0.006945***
	N*L	2	1.179	2.30	0.122031
	D*N*L	2	0.956	1.86	0.176772
	Error	24	0.513		
S (mg·g-1 DW)	***D***	***1***	***47.0825***	***65.692***	***0.000000***
	N	***2***	***4.2695***	***5.957***	***0.007931***
	L	***1***	***18.0059***	***25.123***	***0.000040***
	D*N	2	0.2003	0.280	0.758573
	D*L	1	1.7645	2.462	0.129731
	N*L	***2***	***4.0165***	***5.604***	***0.010066***
	D*N*L	***2***	***3.4009***	***4.745***	***0.018345***
	Error	24	0.7167		

Table S5: Statistical Test ANOVA for photosynthetic pigments

Variable	Source of variation	d.g.	M.S.	F	p
Cl a (mg·g-1 DW)	D	1	0.04711	1.2876	0.267699
	N	***2***	***0.18558***	***5.0726***	***0.014541***
	L	1	0.00708	0.1936	0.663845
	D*N	2	0.03322	0.9079	0.416762
	D*L	1	0.00277	0.0756	0.785733
	N*L	2	0.08510	2.3261	0.119298
	D*N*L	2	0.00296	0.0808	0.922610
	Error	24	0.03659		
Cl (c1+c2) (mg·g-1 DW)	***D***	***1***	***0.009139***	***6.1319***	***0.020718***
	N	***2***	***0.011186***	***7.5052***	***0.002940***
	L	1	0.000783	0.5255	0.475532
	D*N	2	0.001909	1.2809	0.296106
	D*L	1	0.002120	1.4224	0.244668
	N*L	2	0.002192	1.4706	0.249757
	D*N*L	2	0.000275	0.1846	0.832600
	Error	24	0.001490		
Carotenoids (mg·g-1 DW)	***D***	***1***	***0.058599***	***9.8750***	***0.004413***
	N	***2***	***0.029452***	***4.9633***	***0.015706***
	L	1	0.000030	0.0051	0.943522
	D*N	2	0.002852	0.4806	0.624228
	D*L	1	0.000983	0.1656	0.687622
	N*L	2	0.017619	2.9692	0.070436
	D*N*L	2	0.007777	1.3106	0.288262
	Error	24	0.005934		

Table S6: Statistical Test ANOVA for biochemical parameters

Variable	Source of variation	d.g.	M.S.	F	p
Phenols (mg· g-1 DW)	D	1	0.90182	3.62963	0.068810
	N	**2**	**0.97242**	**3.91379**	**0.033798**
	L	1	0.10179	0.40967	0.528202
	D*N	2	0.48753	1.96219	0.162454
	D*L	1	0.68390	2.75256	0.110112
	N*L	**2**	**0.85917**	**3.45798**	**0.047901**
	D*N*L	***2***	***1.22070***	***4.91309***	***0.016274***
	Error	24	0.24846		
	D	1	0.22	2.1	0.156516
	N	2	0.02	0.2	0.850152
	L	1	0.06	0.6	0.454430

ABTS (µmol TE· g-1 DW)	D*N	2	0.07	0.7	0.509075
	D*L	1	0.27	2.6	0.116641
	N*L	2	0.20	2.0	0.163073
	D*N*L	2	0.10	1.0	0.386820
	Error	24	0.10		
DPPH (EC50) (mg·mL-1)	D	1	0.0151	0.064	0.801851
	N	2	0.1308	0.558	0.579559
	L	1	0.4976	2.123	0.158052
	D*N	2	0.0426	0.182	0.834848
	D*L	***1***	***2.7632***	***11.790***	***0.002170***
	N*L	2	0.0404	0.172	0.842760
	D*N*L	2	0.3478	1.484	0.246828
	Error	24	0.2344		
DPPH ((µmol TE· g-1 DW)	***D***	***1***	***47.729***	***15.1792***	***0.000685***
	N	2	0.291	0.0926	0.911912
	L	***1***	***20.003***	***6.3614***	***0.018706***
	D*N	2	0.942	0.2996	0.743838
	D*L	1	0.018	0.0056	0.940884
	N*L	2	0.879	0.2794	0.758666
	D*N*L	2	0.414	0.1317	0.877213
	Error	24	3.144		
Soluble proteins (mg· g-1 DW)	D	***1***	***1298.332***	***909.480***	***0.000000***
	N	2	22.762	15.944	0.000039
	L	1	3.485	2.442	0.131251
	D*N	***2***	***15.853***	***11.105***	***0.000385***
	D*L	1	4.414	3.092	0.091419
	N*L	2	0.277	0.194	0.824719
	D*N*L	2	1.697	1.189	0.321971
	Error	24	1.428		
Lipids (%)	***N***	***2***	***12.9917***	***22.083***	***0.000095***
	L	***1***	***13.8986***	***23.624***	***0.000391***
	N*L	2	2.1766	3.700	0.056024
	Error	12	0.5883		
Carbohydrates (mg· g-1 DW)	D	***1***	***6525.3***	***16.474***	***0.000454***
	N	2	892.1	2.252	0.126939
	L	1	558.3	1.410	0.246750
	D*N	2	94.1	0.237	0.790444
	D*L	***1***	***4911.9***	***12.401***	***0.001747***
	N*L	2	551.5	1.392	0.267874
	D*N*L	2	555.7	1.403	0.265340
	Error	24	396.1		

Appendix 2- Pearson's correlation

	ABTS (µmol ET/g DW)	Proteins (mg/g DW)	% Lipids	EC50 (mg/mL DW)	DPPH (µmol TE/g DW)	Phenols (mg/g DW)	Carbohydrates (mg/g DW)	Chl a (ug/ml)	Chl (c1+c2) (ug/ml)	Carotenoids (ug/ml)
ABTS (µmol ET/g DW)	1.00	0.25	0.09	0.03	-0.20	0.14	0.08	0.25	***0.35***	0.20
Proteins (mg/g DW)	0.25	1.00	***-0.55***	0.02	***-0.50***	0.21	***0.44***	-0.10	***0.40***	***-0.35***
% Lipids	0.09	***-0.55***	1.00	0.22	***0.35***	-0.20	-0.27	0.29	-0.06	***0.43***
EC50 (mg/mL DW)	0.03	0.02	0.22	1.00	***-0.35***	-0.17	0.14	0.06	-0.07	0.16
DPPH (µmol TE/g DW)	-0.20	***-0.50***	***0.35***	***-0.35***	1.00	0.01	***-0.41***	0.15	-0.13	0.30
Phenols (mg/g DW)	0.14	0.21	-0.20	-0.17	0.01	1.00	0.32	0.13	0.26	0.17
Carbohydrates (mg/g DW)	0.08	***0.44***	-0.27	0.14	***-0.41***	0.32	1.00	***-0.39***	-0.12	***-0.44***
Chl a (ug/ml)	0.25	-0.10	0.29	0.06	0.15	0.13	***-0.39***	1.00	***0.81***	***0.82***
Chl (c1+c2) (ug/ml)	***0.35***	***0.40***	-0.06	-0.07	-0.13	0.26	-0.12	***0.81***	1.00	***0.51***
Carotenoids (ug/ml)	0.20	***-0.35***	***0.43***	0.16	0.30	0.17	***-0.44***	***0.82***	***0.51***	1.00
C (mg/g DW)	-0.20	-0.32	***0.42***	0.03	***0.55***	-0.19	-0.24	0.16	-0.13	0.23
N (mg/g DW)	0.25	***0.64***	-0.05	0.18	-0.13	0.13	0.06	0.33	***0.58***	0.10
C/N (mg/g DW)	-0.14	***-0.60***	0.21	-0.18	0.17	-0.17	-0.25	-0.16	***-0.43***	0.04
S (mg/g DW)	-0.12	***-0.59***	***0.43***	0.05	***0.52***	***-0.34***	-0.31	0.07	***-0.34***	0.24
Growth rate (%/día)	-0.28	-0.17	-0.15	-0.02	-0.12	0.05	-0.01	0.18	0.17	0.09
Fv/Fmnight	0.01	0.07	0.07	***0.35***	-0.06	0.08	0.26	0.03	-0.05	-0.05
αETR	0.18	***0.44***	-0.19	-0.04	***-0.44***	-0.03	***0.35***	-0.03	0.22	-0.23
Ek	0.29	0.33	-0.11	-0.12	-0.06	***0.38***	0.15	0.03	0.29	-0.06
ETRmax/NPQmax	0.23	***0.71***	***-0.49***	-0.24	***-0.42***	***0.39***	***0.38***	-0.08	***0.35***	-0.28
Fv/Fm15min	-0.02	-0.24	***0.42***	0.02	***0.40***	-0.22	-0.32	0.27	0.10	0.24
ETRinsitu	***-0.37***	0.09	-0.21	-0.09	-0.02	0.06	0.15	-0.16	-0.11	-0.13

	C (mg/g DW)	N (mg/g DW)	C/N (mg/g DW)	S (mg/g DW)	Growth rate (%/día)	Fv/Fmnight	αETR	Ek	ETRmax/NPQmax	Fv/Fm15min	ETRinsitu
ABTS (µmol ET/g DW)	-0.20	0.25	-0.14	-0.12	-0.28	0.01	0.18	0.29	0.23	-0.02	***-0.37***
Proteins (mg/g DW)	-0.32	***0.64***	***-0.60***	***-0.59***	-0.17	0.07	**0.44**	0.33	***0.71***	-0.24	0.09
% Lipids	***0.42***	-0.05	0.21	***0.43***	-0.15	0.07	-0.19	-0.11	***-0.49***	***0.42***	-0.21
EC50 (mg/mL DW)	0.03	0.18	-0.18	0.05	-0.02	**0.35**	-0.04	-0.12	-0.24	0.02	-0.09
DPPH (µmol TE/g DW)	***0.55***	-0.13	0.17	***0.52***	-0.12	-0.06	**-0.44**	-0.06	***-0.42***	***0.40***	-0.02
Phenols (mg/g DW)	-0.19	0.13	-0.17	***-0.34***	0.05	0.08	-0.03	***0.38***	***0.39***	-0.22	0.06
Carbohydrates (mg/g DW)	-0.24	0.06	-0.25	-0.31	-0.01	0.26	**0.35**	0.15	***0.38***	-0.32	0.15
Chl a (ug/ml)	0.16	0.33	-0.16	0.07	0.18	0.03	-0.03	0.03	-0.08	0.27	-0.16
Chl (c1+c2) (ug/ml)	-0.13	***0.58***	***-0.43***	***-0.34***	0.17	-0.05	0.22	0.29	***0.35***	0.10	-0.11
Carotenoids (ug/ml)	0.23	0.10	0.04	0.24	0.09	-0.05	-0.23	-0.06	-0.28	0.24	-0.13
C (mg/g DW)	1.00	-0.02	0.19	***0.66***	-0.15	-0.11	**-0.42**	-0.06	-0.32	0.22	-0.23
N (mg/g DW)	-0.02	1.00	***-0.87***	-0.21	-0.13	0.09	0.13	0.29	0.29	0.28	-0.14
C/N (mg/g DW)	0.19	***-0.87***	1.00	0.23	0.01	-0.27	-0.11	-0.23	-0.22	-0.26	0.09
S (mg/g DW)	***0.66***	-0.21	0.23	1.00	-0.25	-0.01	**-0.47**	-0.18	***-0.54***	0.32	***-0.37***
Growth rate (%/día)	-0.15	-0.13	0.01	-0.25	1.00	0.12	-0.01	-0.20	-0.09	-0.12	0.20
Fv/Fmnight	-0.11	0.09	-0.27	-0.01	0.12	1.00	0.31	-0.25	-0.02	-0.04	-0.12
αETR	***-0.42***	0.13	-0.11	***-0.47***	-0.01	0.31	1.00	-0.25	***0.51***	-0.32	0.03
Ek	-0.06	0.29	-0.23	-0.18	-0.20	-0.25	-0.25	1.00	***0.50***	-0.05	0.01
ETRmax/NPQmax	-0.32	0.29	-0.22	***-0.54***	-0.09	-0.02	**0.51**	***0.50***	1.00	***-0.48***	0.12
Fv/Fm15min	0.22	0.28	-0.26	0.32	-0.12	-0.04	-0.32	-0.05	***-0.48***	1.00	-0.01
ETRinsitu	-0.23	-0.14	0.09	***-0.37***	0.20	-0.12	0.03	0.01	0.12	-0.01	1.00

MINISTERIO
PARA LA TRANSICION ECOLOGICA
Y EL RETO DEMOGRÁFICO

SECRETARÍA DE ESTADO DE MEDIO AMBIENTE

DIRECCIÓN GENERAL DE BIODIVERSIDAD, BOSQUES Y DESERTIFICACIÓN

Ref: SGPM/BDM/AUTSPP/21/2021

AUTORIZACIÓN ADMINISTRATIVA PARA LA EXTRACCIÓN DE ALGA EXÓTICA INVASORA Y EL SEGUIMIENTO DE SUS POBLACIONES EN LAS COSTAS ESPAÑOLAS POR RAZONES DE INVESTIGACIÓN

La Universidad de Málaga (UMA) tiene previsto realizar la recogida de muestras de la especie exótica invasora de alga parda *Rugulopteryx okamurae* y el seguimiento de sus poblaciones en las costas situadas frente a las provincias de Cádiz, Málaga y Almería, en el marco del proyecto de investigación "Fotoprotectores de algas marinas: cosmecéuticos compatibles con el océano (FACCO)" de la convocatoria de concesión de ayudas a proyectos de I+D+i del Programa Operativo FEDER Andalucía 2014-2020. El objetivo de este proyecto es realizar un estudio botánico-ecológico y ecofisiológico de la especie, así como valorar sus compuestos bioactivos y su uso en cosmecéuticos compatibles con el medio ambiente para obtener un producto cosmético natural con alta capacidad fotoprotectora-antioxidante e inmunoestimulante.

Estos trabajos de investigación implican la realización de una serie de muestreos de la especie para llevar a cabo análisis moleculares, genéticos y estudios *in vivo* en laboratorio de caracteres morfológicos, fotosintéticos y reproductivos, y también la elaboración de pliegos de herbario (a depositar en el Herbario-MGC de la UMA). Además, requieren el seguimiento periódico de las poblaciones de *R. okamurae* en las zonas de estudio, así como la caracterización y la valoración del estado ecológico de las comunidades afectadas por el alga invasora (*Posidonia oceanica*, *Cymodocea nodosa, Cystoseira* spp., entre otras). Los parámetros estudiados se analizarán de forma simultánea en las poblaciones de *R. okamurae* y en las comunidades nativas afectadas y/o próximas a estas, y siempre haciendo uso de técnicas no invasivas: técnica de cuadrantes establecida por Cebrián *et al.* (2000) para la cobertura de especies invasoras (Otero *et al.*, 2013), y transectos de 20 m paralelos a la línea de costa por el método del intercepto-lineal, para el seguimiento de las praderas de fanerógamas.

La investigación *in situ* de las poblaciones a muestrear se realizará de dos a cuatro veces al año (invierno-verano o en cada estación), en las siguientes zonas de muestreo, entre la isobata de los 15 m y los puntos de muestreo especificados para cada zona:

Pl. San Juan de la Cruz, 10
28071-MADRID

Localización de zonas de muestreo y su coincidencia con espacios de la Red Natura 2000 marina de competencia estatal y los tipos de hábitat de interés comunitario

	PUNTOS DE MUESTREO	COORD X	COORD Y	EMP & THIC
PARQUE NATURAL DEL ESTRECHO-TARIFA	1. Parque natural del Estrecho-Tarifa	36º 0' N	5º 36' O	
NERJA-MARO	1. Nerja-Maro	36.749417	-3.864694	ZEPA *Bahía de Málaga-Cerro Gordo*
	2. Nerja-Maro	36.751619	-3.835667	ZEPA *Bahía de Málaga-Cerro Gordo*
MARBELLA-ESTEPONA	1. Marbella-Estepona	36.483161	-4.737819	
	2. Marbella-Estepona	36.483925	-4.726811	
ALMERÍA-ROQUETAS DE MAR	1. Faro de Roquetas de Mar	36.752919	-2.605068	a escasos metros de THIC 1120 (*Posidonia oceanica*) THIC 1110 (*Cymodocea nodosa*)
	2. Playa Aguadulce	36.811665	-2.566222	
	3. Roquetas de Mar	36.688138	-2.657732	ZEC *Fondos Marinos de Punta Entinas-Sabinar* *ZEPA Bahía de Almería* THIC 1120
	4. Roquetas de Mar	36.793887	- 2.584018	ZEC *Arrecifes de Roquetas de Mar* *ZEPA Bahía de Almería* THIC 1120

Las zonas de muestreo 3 y 4 de Roquetas de Mar se localizan respectivamente en aguas de la zona especial de conservación (ZEC) ES6110009 *Fondos Marinos de Punta Entinas-Sabinar* y la ZEC ES6110019 *Arrecifes de Roquetas de Mar,* declaradas mediante *Orden AAA/1366/2016, de 4 de agosto, por la que se declaran zonas especiales de conservación de lugares de importancia comunitaria de la Región Marina Mediterránea de la Red Natura 2000, se aprueban sus correspondientes medidas de conservación y se propone la ampliación de los límites geográficos de dos lugares de importancia comunitaria,* debido a la presencia de los tipos de hábitat de interés comunitario *1110 Bancos de arena cubiertos permanentemente por agua marina, poco profunda* y *1120 *Praderas de Posidonia (Posidonion oceanicae*), incluidos en el Anexo I de la Directiva Hábitats, y de la nacra (*Pinna nobilis*), especie incluida en el anexo II del Convenio de Berna y en el anexo II del Convenio de Barcelona, en el anexo V de la *Ley 42/2007, de 13 de diciembre*, y en listados y catálogos de protección, tanto estatal como autonómicos. Además, estas dos zonas de muestreo se encuentran localizadas en la zona de especial protección para las aves (ZEPA) *Bahía de Almería,* que constituye una importante área de alimentación de la pardela balear (*Puffinus mauretanicus*) y la gaviota de Audouin (*Larus audouinii*). Asimismo, existen colonias importantes de gaviota picofina (*Larus genei*), de charrán común (*Sterna hirundo*) y de charrancito común (*Sterna albifrons*) en los humedales costeros adyacentes. Por otro lado, las zonas de muestreo 1 y 2 de Nerja-Maro se encuentran en aguas de la ZEPA ES7010020 *Bahía de Málaga-Cerro Gordo,* una importante zona marina de concentración de gaviota cabecinegra (*Larus melanocephalus*), en los meses de invierno, y de pardela balear (*Puffinus mauretanicus*), que utiliza la zona como área de alimentación y descanso.

Estos trabajos implican la manipulación, recolección, transporte, custodia y estudio en laboratorio de ejemplares de *Rugulopteryx okamurae,* especie incluida en el *Catálogo Español de Especies Exóticas Invasoras*, creado a través del artículo 64 de la *Ley 42/2007, de 13 de diciembre, del Patrimonio Natural y de la Biodiversidad* y regulado por el *Real Decreto 630/2013, de 2 de agosto.*

ESTA DIRECCIÓN GENERAL, en virtud de la competencia estatal sobre biodiversidad marina establecida en el artículo 6 de *la Ley 42/2007, de 13 de diciembre, del Patrimonio Natural y de la Biodiversidad*, conforme al artículo 64.5 de dicha ley y el artículo 7.1 del *Real Decreto 630/2013, de 2 de agosto, por el que se regula el Catálogo español de especies exóticas invasoras,* y en virtud de las facultades que le han sido conferidas por el *Real Decreto 500/2020, de 28 de abril, por el que se desarrolla la estructura orgánica básica del Ministerio para la Transición Ecológica y el Reto Demográfico*, ha resuelto:

AUTORIZAR

a D. Félix López Figueroa (DNI 42795556-P) y Dª. Nathalie Korbee Peinado (DNI 25717338-A), para llevar a cabo los trabajos descritos en aguas bajo soberanía o jurisdicción española de la demarcación marina del Estrecho y Alborán, que implican el manejo, la recogida, el transporte y la custodia de ejemplares de *Rugulopteryx okamurae*, con los siguientes requerimientos:

- el área geográfica de los trabajos se limitará a los puntos de muestreo especificados, en los siguientes tramos de costa de la demarcación marina del Estrecho y Alborán: Tarifa (Cádiz), Marbella-Estepona y Nerja-Maro (Málaga) y Almería-Roquetas de Mar (Almería);
- será de obligado cumplimiento lo establecido en el artículo 64.5 de la *Ley 42/2007, de 13 de diciembre*, y en el artículo 7 del *Real Decreto 630/2013, de 2 de agosto,* en relación a los efectos de la inclusión de una especie en el *Catálogo Español de Especies Exóticas Invasoras*, con la siguiente excepción:
    - posesión y transporte de ejemplares vivos, de sus restos o propágulos que pudieran sobrevivir o reproducirse, por razones de investigación, salud o seguridad de las personas;
- la investigación *in situ* de las poblaciones a muestrear se realizará de 2 a 4 veces al año;
- la prospección se realizará en contra del gradiente de presencia del alga para evitar que el buzo sea un vector de propagación -o incluso de introducción- de la especie en el medio natural;
- la recolección de las muestras se realizará a mano, sin guantes o con pinzas, mediante inmersiones con escafandra autónoma de buceo;
- se extraerá del medio únicamente el material estrictamente necesario para la consecución de los objetivos de la investigación;
- los talos extraídos serán depositados inmediatamente en bolsas plásticas de cierre hermético;
- no se realizarán liberaciones al medio natural bajo ninguna circunstancia;
- tanto los trabajos de muestreo como los de seguimiento de las poblaciones se realizarán manteniendo un control estricto de la flotabilidad, con aproximaciones lentas y progresivas para evitar desprender talos de manera accidental con el equipo de buceo y/o materiales de muestreo; en caso de que esto ocurriera se recogerá dicho material y se introducirá rápidamente en una bolsa de cierre hermético;

- se evitarán al máximo las perturbaciones sobre las comunidades y el hábitat circundante derivadas de la recolección de ejemplares y del estudio *in situ* de las comunidades;
- las muestras recolectadas se mantendrán en condiciones adecuadas que permitan su apropiado transporte a, y mantenimiento en, las instalaciones de la Facultad de Ciencias de la UMA;
- las muestras se analizarán en los laboratorios del Departamento de Ecología y Geología y del Departamento de Botánica y Fisiología Vegetal de la Facultad de Ciencias de la UMA y en las instalaciones acuícolas del Instituto Universitario de Biotecnología y Desarrollo Azul (IBYDA) en el Centro Experimental Grice Hutchinson de la UMA (San Julián)
- después de cada inmersión todo el equipo de buceo y los materiales empleados para realizar el muestreo y/o el seguimiento de las poblaciones de la especie se guardarán en bolsas y se lavarán con agua e hipoclorito sódico durante cinco minutos, con especial atención en las piezas de neopreno y quitando manualmente los restos de la especie que hayan podido quedar adheridos, y deberá permanecer en cuarentena preventiva por al menos 48 horas antes de poder ser utilizado de nuevo en otra localidad de buceo;
- los utensilios de laboratorio que hayan estado en contacto con el alga serán higienizados con una solución de hipoclorito sódico al (4%);
- los tanques de experimentación serán tratados con una disolución de cloro líquido al 13%, a razón de 1 litro de producto por cada 10 m³ de agua, durante 24 horas antes de proceder al su vaciado;
- las muestras sobrantes tras los experimentos solo se podrán desechar en condiciones controladas de laboratorio y tras ser sometidas a un proceso de destrucción química (baño en solución de hipoclorito sódico) y/o calórica (ciclo de autoclave;
- la coordinación de los trabajos de recolección y de seguimiento en el mar correrá a cargo de los dos investigadores autorizados D. Félix López Figueroa y Dª. Nathalie Korbee Peinado;
- se utilizará la embarcación *MOBULA*, con matrícula 6ª-GR-1-2-15 y capacidad para 14 personas, tripulación incluida;
- para el transporte de las muestras recolectadas al laboratorio se utilizará uno de los siguientes vehículos: *Citroen Jumpy* con matrícula MA-1892-CW, *Renault Kangoo* con matrícula 6415-LCR, *Renault Trafic* con matrículas 4698-GTZ o 7296-GLF, o *VW LT35* con matrícula 7498-CYJ;
- deberá comunicarse con suficiente antelación a la Subdirección General de Biodiversidad Terrestre y Marina (bzn-biomarina@miteco.es) cualquier cambio que sea necesario para el desarrollo de los trabajos respecto a lo informado en relación a la embarcación o personal autorizados, así como el motivo del mismo, y se deberá facilitar la información correspondiente para su adecuada identificación, no estando autorizados a dicho cambio hasta que reciban una repuesta afirmativa a dicha comunicación;
- para el desarrollo de los mencionados trabajos se antepondrá en todo momento el buen estado de las condiciones ambientales del entorno.

En todo caso, se deberá contar y/o cumplir con las correspondientes licencias, trámites, obligaciones y autorizaciones de orden administrativo, fiscal, sanitario o laboral exigidos por la legislación vigente, así como el correspondiente permiso de acceso a terrenos de titularidad privada y pública de otras instituciones.

Deberá tenerse en cuenta que, si la finalidad de la recolección de muestras biológicas fuera el acceso para utilización de recursos genéticos, pudiera ser de aplicación el *Real Decreto 124/2017, de 24 de*

febrero, relativo al acceso a los recursos genéticos procedentes de taxones silvestres y al control de la utilización.

No se podrán facilitar estas muestras biológicas a ninguna persona no autorizada y, en todo caso, la transmisión de las muestras biológicas a terceros se realizará en las mismas condiciones que las impuestas en esta autorización. En particular, si la cesión a terceros tiene como finalidad la utilización de recursos genéticos, puede requerir una autorización de acceso a recursos genéticos en virtud del *Real Decreto 124/2017, de 24 de febrero, relativo al acceso a los recursos genéticos procedentes de taxones silvestres y al control de la utilización*.

La difusión por redes sociales de cualquier material audiovisual obtenido mediante la presente autorización deberá contar previamente con el visto bueno de la Subdirección General de Biodiversidad Terrestre y Marina. Para ello deberán ponerse en contacto a través de la dirección de correo electrónico bzn-biomarina@miteco.es adjuntando el contenido que se pretende compartir e indicando la red o redes que se prevé utilizar.

La presente autorización será válida **desde la firma de la misma y hasta el 30 de abril de 2022.**

Deberá remitirse a la Subdirección General de Biodiversidad Terrestre y Marina (bzn-biomarina@miteco.es), en un plazo que no excederá de tres meses a partir del vencimiento de la validez de esta autorización, un informe con los resultados obtenidos en las actuaciones desarrolladas amparándose en la presente autorización, incluyendo:

- registro de sitios inspeccionados y consultados con presencia/ausencia de la especie;
- registro de datos de seguimiento periódico de las poblaciones de *Rugulopteryx okamurae,* en las zonas de estudio;
- registro de datos periódicos de caracterización y de valoración del estado ecológico de las comunidades afectadas por *Rugulopteryx okamurae,* en las zonas de estudio;
- relación de ejemplares recolectados en cada salida (fecha, número de ejemplares, estado del ejemplar, medidas) por zona de extracción (coordenadas geográficas UTM);
- descripción de los trabajos e incidencias, en su caso.

Los datos que se incluyan en este informe deberán presentarse en el formato que se determine por la Subdirección General de Biodiversidad Terrestre y Marina de manera que puedan integrarse en el Sistema Integrado de Información del Banco de Datos de la Naturaleza.

Asimismo, deberá remitirse a la Subdirección General de Biodiversidad Terrestre y Marina copia de cualquier material científico-técnico y educativo desarrollado amparándose en la presente autorización. En todo caso, para la publicación o divulgación, por cualquier medio de comunicación, de información relativa al proyecto y/o de imágenes tomadas durante el mismo, se deberá señalar que esta ha sido obtenida tras solicitar autorización del Ministerio para la Transición Ecológica y el Reto Demográfico.

El incumplimiento de los preceptos indicados en esta autorización que puedan infringir lo establecido en la materia en la *Ley 42/2007, de 13 de diciembre*, podrá generar responsabilidad de naturaleza

administrativa de acuerdo a lo establecido en el Título VI, sin perjuicio de la exigible en vía penal, civil o de otro orden en que puedan incurrir. La inobservancia de cualquiera de los preceptos indicados anteriormente podrá suponer la revocación de la presente autorización y la no autorización de nuevas solicitudes.

En el ámbito de la Administración General del Estado, contra la resolución de la presente autorización podrá interponerse recurso de alzada ante el Secretario de Estado de Medio Ambiente en el plazo de un mes de acuerdo con lo previsto en los artículos 121 y 122 de la *Ley 39/2015, de 1 de octubre.*

EL SUBDIRECTOR GENERAL DE BIODIVERSIDAD,
TERRESTRE Y MARINA

(Firma electrónica)

Miguel Aymerich Huyghues-Despointes