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# Seasonal photoacclimation patterns in the intertidal macroalga *Cystoseira tamariscifolia* (Ochrophyta)

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**Summary**: *Cystoseira tamariscifolia* thalli collected from rocky shores and rockpools in winter and summer in Southern Spain were incubated for 7 days in UV transparent cylindrical vessels under outdoor conditions. Photosynthetic activity estimated as in vivo chlorophyll *a* fluorescence of photosystem II, photosynthetic pigments, antioxidant activity (DPPH assay), phenolic compounds and total internal C and N contents were determined after short-term (3 d) and mid-term (7 d) periods. Maximum quantum yield of PSII ($F_v/F_m$) was significantly higher in field-collected algae and after 7 d incubation in winter than in summer. In rocky shores and rockpools thalli, maximum electron transport rate ($ETR_{max}$) and photosynthetic efficiency ($\alpha_{ETR}$) were much higher in summer than in winter. ETR of outdoor-grown thalli (in situ ETR) showed a daily pattern, with a decrease at noon in both winter and summer (3rd and 7th days). We found much higher antioxidant activity in thalli collected in summer than in winter. However, the concentration of internal UV screen substances (polyphenols) was higher in winter than in summer, whereas the release of phenolic compounds was lower. The highest capacity of acclimation in *C. tamariscifolia* found in summer and RS with emersion periods was explained by the highest dynamic photoinhibition, energy dissipation (non-photochemical quenching) and antioxidant activity ($EC_{50}$).

**Keywords**: antioxidant activity; *Cystoseira tamariscifolia*; phenolic compounds; photoinhibition; photoprotection.

**Patrones estacionales de fotoaclimatación en el alga intermareal, *Cystoseira tamariscifolia* (Ochrophyta)**

**Resumen**: Talos de *Cystoseira tamariscifolia* recolectados en pozas y plataformas rocosas intermareales (Sur de España) en invierno y en verano se incubaron bajo radiación solar durante 7 días en recipientes cilíndricos de metacrilato transparentes a la radiación UV. Se estimó la actividad fotosintética a través de la fluorescencia de la clorofila *a* asociada al fotosistema II, el contenido de pigmentos fotosintéticos y compuestos fenólicos, actividad antioxidante y el contenido total en C y N internos tras 3 y 7 días de incubación. Los valores iniciales del rendimiento cuántico máximo ($F_v/F_m$) fueron significativamente mayores en algas recolectadas en invierno que en verano mientras que la tasa de transporte electrónico máximo ($ETR_{max}$) y la eficiencia fotosintética fueron mayores en verano que en invierno en ambas zonas. Por otra parte, la tasa de transporte electrónico determinada bajo radiación solar presentó un patrón diario, con una disminución a mediodía, tanto en invierno como en los períodos de verano. La actividad antioxidante fue mayor en algas recogidas en verano; sin embargo, la concentración interna de compuestos fenólicos fue mayor en invierno que en verano, mientras que en la tasa de excreción se observó lo contrario. La alta capacidad de aclimatación en *C. tamariscifolia* en algas sometidas a emersión en las plataformas rocosas en verano se explica por su alta fotoinhibición dinámica, su capacidad de disipación de energía (amortiguamiento no fotoquímico, NPQ) y su actividad antioxidante ($EC_{50}$).

**Palabras clave**: actividad antioxidante; *Cystoseira tamariscifolia*; compuestos fenólicos; fotoinhibición; fotoprotección.

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## INTRODUCTION

Macroalgae in temperate regions, such as the Mediterranean coast of Spain, are exposed to high daily integrated solar irradiance, both ultraviolet (UV) and photosynthetically active (PAR) (Häder and Figueroa 1997). The high irradiance and transparency of shallow water in this region suggest that macroalgae have de-

veloped efficient photoprotection mechanisms to tolerate light stress (Figueroa and Gómez 2001). In fact, intertidal macroalgae subject to high solar irradiance and desiccation can survive and grow under the stressful conditions of the intertidal system due to active photoprotection mechanisms such as dynamic photoinhibition, accumulation of UV screen substances and increase in antioxidant capacity (Häder and Figueroa 1997, Korbee et al. 2006, Bischof et al. 2006, Hanelt and Figueroa 2012).

Brown algae accumulate UV screen compounds (polyphenols) under high PAR and UVR (Pavia et al. 1997). In addition to the UV screen capacity, phenolic compounds also have strong antioxidant activity (Connan et al. 2006, Cruces et al. 2012), thus reducing DNA damage (Gómez and Huovinen 2010). Phlorotannins are phenolic compounds identified in brown algae constituting up to 25% dry weight (Targett et al. 1992). Concentrations of phlorotannins show phenotypic plasticity in response to changes in environmental parameters, such as salinity, nutrients, light quality and irradiance availability, and herbivory (Peckol et al. 1996, Pavia et al. 1997, Pavia and Toth 2000, Honkanen and Jormalainen 2002, Swanson and Druehl 2002, Abdala-Díaz et al. 2006). Phenolic compounds can also be released from the thalli into alkaline medium of seawater under stressful conditions, reacting rapidly with both proteinaceous and carbohydrate substances to form UV-absorbing complexes (Dujmov et al. 1996, Swanson and Druehl 2002, Koivikko et al. 2005). Hence, it is very important to determine both internal and released polyphenols in order to evaluate the photoprotective capacity of these compounds (Koivikko et al. 2005, Gómez and Huovinen 2010, Cruces et al. 2012).

*Cystoseira* species are considered to grow mainly in high-quality waters according to the Water Framework Directive of the European Union (WFD, 2000/60/EC) and they are indicator of waters with high-quality ecological status including the Andalusia Coast (Ballesteros et al. 2007, Arévalo et al. 2007). In our study, *Cystoseira tamariscifolia* (Hudson) Papenfuss, was selected as a model species because it is an abundant and is a key species on the southern shores of the Mediterranean Sea. The evaluation of photosynthetic and antioxidant activities in thalli collected at different places in short spatial scales such as rocky shores (RS, thalli exposed to the air during some time during the daily cycle) and rockpools (RP, thalli always immersed in the seawater and with a high rate of water renewal) can give information on the vulnerability and acclimation capacity of this species to environmental changes. In addition, it is important to analyse the relation between photosynthetic activity and energy dissipation by using *in vivo* chlorophyll *a* fluorescence and polyphenol content and antioxidant activity in thalli collected in summer and winter and submitted to an emersion/immersion regime (RS- versus RP-collected thalli) and incubated under outdoor conditions.

Two physiological indicators have been used to evaluate the physiological status of seaweeds (Figueroa and Korbee 2010): (1) maximum quantum yield of PSII ($F_v/F_m$) as an indicator of physiological status of macroalgae and photoinhibition (Schreiber et al. 1986) and (2) electron transport rate (ETR) as an indicator of photosynthetic capacity (Figueroa et al. 2003). Two biochemical indicators of stress conditions have also been used: (1) stoichiometric ratios (C:N) as an indicator of nutritional status and (2) the content of phenolic compounds, such as photoprotective and antioxidant substances in brown algae (Pavia et al. 1997, Connan et al. 2004, Abdala-Díaz et al. 2006).

The hypothesis is that thalli submitted to high stress conditions in the natural environment have a greater acclimation capacity and less vulnerability to increased solar irradiance in the short to medium term (3 and 7 days).

## MATERIALS AND METHODS

### Species and sampling

*C. tamariscifolia* (Hudson) Papenfuss, (Phaeophyceae: Fucales) was randomly collected in winter (February, 2011) and summer (June and July, 2011) at La Araña beach, Málaga, southern Spain (36°42'N, 4°19'W) in the morning (before 11:00 am local time). The samples were collected in RS (areas in high zones of the platform) and in RP (with a high rate of water renewal). RS-collected thalli are exposed to air during low tide, when they are subjected to temperature and desiccation stress, while RP-collected thalli are always submerged but may be exposed to temperature stress when the pool is isolated from the sea during low tide. Thalli were transported under cold conditions to the laboratory in order to avoid damage to the biological material. Rocky shores and rockpools are very close to each other (a distance of less than 1.0-1.5 m). Samples for biochemical analysis were frozen in situ using liquid nitrogen.

### Experimental design

*C. tamariscifolia* were acclimated for 48 h in a polyvinyl methacrylate UV transparent vessel (Plexiglass XT- 29080) with a final volume of 1.5 L seawater covered with two layers of neutral density filters to remove 65% of full solar radiation (PAR+UVA+UVB, mesh with pore size 1 $mm^2$) in order to reduce the risk of photoinhibition during the acclimation period in the experimental vessels. Twelve cylindrical vessels with 250 g of thalli were placed in tanks of 250 L with circulating fresh water to control the temperature of the system. After the acclimation time, thalli were incubated to full solar radiation in the same outdoor systems located on the roof of the building of the Central Services for Research (University of Malaga) for 7 days. The experiment was performed in both winter (from 8 to 16 February 2011) and summer (from 27 June to 5 July 2011). Six replicates for thalli collected from RS and another six for those collected from RP were used in each period. Seawater was N-enriched at the beginning of the experiment with $NaNO_3$ reaching a maximum final concentration of 50 μM $NO_3^-$. Seawater was renewed and N-enriched in the experimental vessels after

3 days. The incubation temperature reached maximum temperature values of 18°C in winter and 22°C in summer, with temperature oscillations during the day and night of 2-3°C in winter and 1-2°C in summer. The temperature was maintained by using a cooling unit Titan-500 (Aqua Medic, Bissendorf, Germany) with a submersible pump for water circulation (Ocean runner OR Aqua Medic, Bissendorf, Germany). The temperature was measured using a HOBO U22 Water Temp Pro v2 logger (Onset Computer Corporation, Massachusetts, USA). Algae were continuously aerated inside the cylinders using a 3010-1 HPEMODEL air pump (HPE Technology, Barcelona, Spain). Measurements of photosynthetic parameters and biochemical analysis were done in field-collected algae and after 3 and 7 days of incubation. Samples for biochemical analysis were stored at –80°C until analysis.

Incident solar irradiance of PAR (400-700 nm), UVA (320-400 nm) and UVB (280-320 nm) was measured continuously in air using an NILU-6 UV-PAR Multifilter radiometer (Geminali AS, Oslo, Norway). The irradiances of UVA and UVB were calculated from the data of the different UV filters according to Høiskar et al. (2003).

### Photosynthesis and energy dissipation by using in vivo chlorophyll *a* fluorescence

In order to conduct rapid light curves (RLCs) according to Schreiber et al. (1995), apical algal pieces were collected from each treatment in field-collected algae and after 3 and 7 days of incubation (in the morning) and introduced in incubation chambers with 10 mL seawater at the 12 incremental irradiances (E1=9.3, E2=33.8, E3=76, E4=145, E5=217, E6=301, E7=452, E8=629, E9=947, E10=1403, E11=2084 and E12=3444 μmol photons $m^{-2}$ $s^{-1}$) of white light (halogen lamp provided by the Diving-PAM).

$F_0$ (basal fluorescence from fully oxidized reaction centers of PSII) and $F_m$ (maximum fluorescence from fully reduced PSII reaction centre) were determined after 15 minutes in darkness to obtain the maximum quantum yield ($F_v/F_m$), $F_v$ being the difference between $F_m$ and $F_0$ (Schreiber et al. 1995).

The effective quantum yield ($\Delta F/F_{m'}$) was calculated according to Schreiber et al. (1995):

$$\Delta F/F_{m'} = (F_{m'} - F)/F_{m'} \quad (1)$$

where $F_{m'}$ is the maximum fluorescence induced with a saturating white light (halogen lamp) and $F$ is the current steady-state fluorescence in light-adapted thalli.

The ETR was calculated according to Schreiber et al. (1995) as follows:

$$\text{ETR (μmol electrons } m^{-2} s^{-1}) = \Delta F/F_{m'} \times E \times A \times F_{II} \quad (2)$$

where $E$ is the incident PAR irradiance, $A$, is the absorptance of the thalli of the fraction of incident irradiance estimated using a PAR sensor with a cosine response (Licor 192 SB) according to Figueroa et al. (2009), and $F_{II}$ is the fraction of chlorophyll associated with PSII (400-700 nm) being 0.8 in brown macroalgae (Figueroa et al. 2014). Both maximum ETR ($ETR_{max}$) and the initial slope of ETR versus irradiance curves ($\alpha_{ETR}$) as an estimator of photosynthetic efficiency were obtained from the tangential function reported by Platt and Gallegos (1980).

In addition, to test and characterize the effect of the light quality changes by decreasing the irradiance of the Diving-PAM halogen lamp, i.e. to decrease the proportion of blue light (Hanelt et al. 2003), effective quantum yields were also measured using red light (light-emitted diodes) provided by PAM-2000 or Water PAM fluorometer. No significant differences were found in the effective quantum yield data in RLCs conducted by halogen lamp (Diving-PAM) and red light of PAM-2000 and Water PAM in a wide range of irradiances from 18 to 2200 μmol $m^{-2}$ $s^{-1}$ (data not shown). Thus, the ETR as RLCs was determined after 20 seconds of exposure. In addition, ETR was calculated from the measurements of effective quantum yield using Formula (2) of algae apical parts in the vessels during daily cycles; this ETR to distinguish from the ETR of RLCs is called in situ ETR. Measurements were conducted twice in winter (6:00,8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 GMT), and twice in summer (08:00, 10:00; 12:00, 14:00, 16:00, 18:00 and 20:00 GMT).

Non-photochemical quenching (NPQ) was calculated according to Schreiber et al. (1995) as:

$$NPQ = (F_m - F_{m'})/F_{m'} \quad (3)$$

Maximum NPQ ($NPQ_{max}$) and the initial slope of NPQ ($\alpha_{NPQ}$) versus irradiance curves were obtained from the tangential function of NPQ according to Jassby and Platt (1976).

### Biochemical variables

Total internal carbon and nitrogen contents on a dry weight (DW) basis were determined using a CNHS-932 model element analyser (LECO Corporation, Michigan, USA).

Chlorophyll *a* (Chl *a*) and carotenoids pigments were determined in samples (0.025 g fresh weight) taken in six replicates from field-collected algae and after 3 and 7 days of exposure. Chl *a* and Chl $c_1$+$c_2$ were extracted in 1 mL of 90% acetone neutralized by magnesium carbonate hydroxide and measured in a spectrophotometer (UV Mini-1240 model, Shimazdu, Columbia, USA) using the formula reported by Ritchie (2008).

Total phenolic compounds (polyphenols) were determined using 0.25 g fresh weight (FW). Samples were pulverized in a mortar and pestle with sea-sand using 2.5 mL of 80% methanol. The mixture was kept overnight at 4°C and then centrifuged at 4000 rpm for 15 min and the supernatant was collected. Total phenolic compounds were determined colorimetrically using Folin-Ciocalteu reagent (Folin and Ciocalteu 1927). Phloroglucinol (1,3,5-trihydroxybenzene, Sigma P-3502) was used as a standard. Finally, the absorbance was determined at 760 nm using a Shimadzu UVMini-1240 spectrophotometer (Celis-Plá et al.

2014). Phenolic concentration was expressed as mg $g^{-1}$ DW after determining the fresh to dry weight ratio in the tissue (the ratio was 5.6). The results are expressed as average±standard deviation from six replicates of each treatment.

The release of polyphenols (PR) in the seawater was determined by measuring the optical density in a spectrophotometer (UVMini-1240 model, Shimadzu, Columbia, USA) at the maximum absorption of polyphenols in the seawater, i.e. 270 nm (Ragan and Craigie 1980). The water samples were taken from waters in which *C. tamariscifolia* were growing. The concentration, expressed as mg $g^{-1}$ DW $day^{-1}$, was obtained using phloroglucinol dissolved in seawater as standard. PR was determined after 3 and 7 days of incubation.

The antioxidant activity of seaweed extracts was estimated indirectly using the method based on reducing the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), according to Blois (1958). The same supernatant used for phenolic compounds was used for DPPH analysis. 150 µL of DPPH prepared in 90% methanol (90MeOH: $10H_2O$) were added to each extract. The reaction was complete after 30 min in the dark at room temperature (~20°), and the absorbance was read at 517 nm in a spectrophotometer (UVMini-1240 model, Shimadzu, Columbia, USA). The calibration curve made with DPPH was used to calculate the remaining concentration of DPPH in the reaction mixture after incubation. Values of DPPH concentration (mM) were plotted against plant extract concentration (mg DW $mL^{-1}$) in order to obtain the oxidation index, $EC_{50}$, which represents the concentration of the extract, expressed as mg DW $mL^{-1}$, required to scavenge 50% of the DPPH in the reaction mixture. Ascorbic acid was used as positive control (Celis-Plá et al. 2014).

### Statistical analysis

The effects of the treatments on the ecophysiological variables were analysed by a three-way ANOVA (Underwood 1997). This test was performed for *C. tamariscifolia* including season, day and thalli origin (RS and RP) as fixed factors. The design allows testing for interactive and additive effects. Homogeneity of variance was tested using the Cochran test and by visual inspection of the residuals. Student Newman-Keuls tests (SNK) were performed after significant ANOVA interactions (Underwood 1997). Analyses were done with SPSS v.21 (IBM, USA).

## RESULTS

### Solar radiation and temperature

The daily integrated irradiance in the air during the experimental period of PAR, UVA and UVB is represented in Figure 1. The daily integrated irradiance in the period from 8 to 16 February 2011 was 0.21 MJ $m^{-2}$ of UVB, 4.53 MJ $m^{-2}$ of UVA and 54.3MJ $m^{-2}$ of PAR, whereas from 27 June to 5 July it was 0.64 MJ $m^{-2}$ of

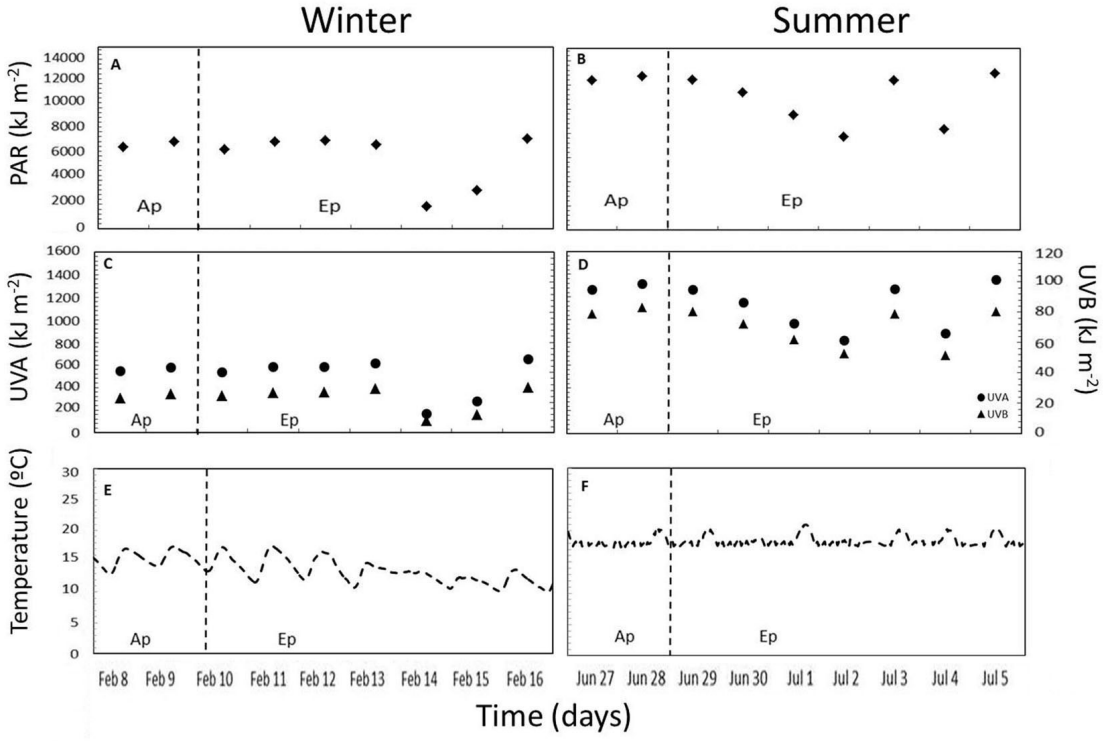


Fig. 1. – Daily integrated irradiance (DIE) in the air expressed as kJ $m^{-2}$ of PAR (400-700 m) (A, B), UVA (320-400 nm) and UVB (280-320 nm) (C, D) in winter (February) (A, C) and summer (July) (B, D) (days). The harvesting of *C. tamariscifolia* was conducted at time 0, then the algae were incubated in 1.5-L cylindrical vessels for 2 days under decreased solar irradiance conditions (acclimation period, AP). After this period, the algae were incubated for 7 d under full solar irradiance (experimental period, EP). Average daily integrated irradiance was calculated from 8 to 16 February in winter and from 27 of June to 5 July in summer. Underwater temperature in the experiment in winter (E) and summer (F)

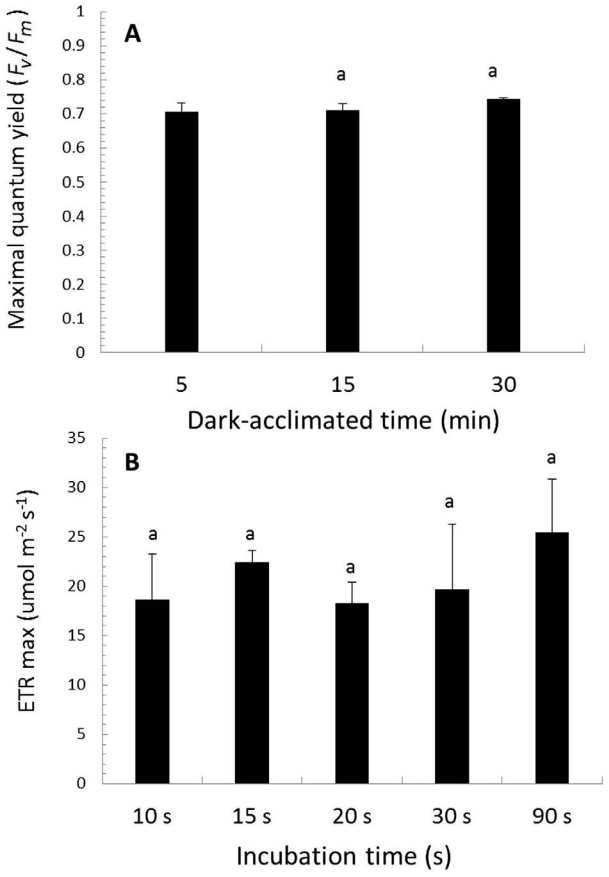


Fig. 2. – Measurement of maximum quantum yield ($F_v/F_m$) after incubation of *C. tamariscifolia* thalli in different time periods in darkness (5, 15 and 30 min) (A). Different incubation time (10, 15, 20, 30 and 90 s) under increased irradiances of actinic light (PAR) to estimate the $ETR_{max}$ (B).

UVB, 10.3 MJ $m^{-2}$ of UVA and 99.8 MJ $m^{-2}$ of PAR; about 3.1 times (UVB), 2.3 times (UVA) and 1.8 times (PAR) higher in summer than in winter (Fig. 1). Most of the time the sky was cloudless in winter experiments except for days 4 and 5 (Fig. 1A, C) whereas, in the summer experiment thin clouds were also observed on days 3 to 5 (Fig. 1B, D). The average underwater temperature in the incubation vessels was maintained at 18°C in winter and 22°C in summer during the day and at 12°C in winter and 17°C in summer during the night (Fig. 1E, F).

## Physiological and biochemical responses

In order to determine the optimal incubation time in darkness to estimate $F_v/F_m$, thalli were incubated in darkness for 5, 15 and 30 min (Fig. 2A). No significant differences were found among the tested times and 15 min was selected as it is the most common dark exposure time found in the literature. In order to determine the optimal incubation time in RLCs to reach steady-state conditions of effective quantum yield and ETR, thalli were incubated under different increased intensities for 10, 15, 20, 30 and 90 seconds of incubation time in each actinic light. No significant differences were found between 20 and 90 seconds (Fig. 2B) and

Table 2. – ANOVA results testing for the effect of Seasons, Time and Origen of algae (RP; RS) on the photosynthetic parameters; maximum quantum yield ($F_v/F_m$), electron transport rate ($ETR_{max}$) exp ressed as μmol electrons $m^{-2}$ $s^{-1}$, photosynthetic efficiency ($\alpha_{ETR}$) as the initial slope of ETR versus irradiance rapid light curves (RLCs), saturated irradiance of ETR ($Ek_{ETR}$, expressed in μmol $m^{-2}$ $s^{-1}$ ) and maximum non-photochemical quenching ($NPQ_{max}$) of *Cystoseira tamariscifolia*; significant differences at α<0.05 are shown in bold.

| | | df | MS | F | p |
|---|---|---|---|---|---|
| $F_v/F_m$ | Season (S) | 1 | 0.054 | 57.58 | **0.00** |
| | Time (T) | 1 | 0.019 | 20.28 | **0.00** |
| | Origen algae (O) | 1 | 0.006 | 6.58 | **0.01** |
| | S×T | 1 | 0.005 | 5.67 | **0.02** |
| | S×O | 1 | 0.002 | 2.51 | 0.12 |
| | T×O | 1 | 0.001 | 0.80 | 0.37 |
| | S×T×O | 1 | 0.001 | 0.62 | 0.43 |
| | Residual | 40 | 0.001 | | |
| $ETR_{max}$ | Season (S) | 1 | 35794.31 | 463.63 | **0.00** |
| | Time (T) | 1 | 5.21 | 0.07 | 0.80 |
| | Origen algae (O) | 1 | 1843.20 | 23.87 | **0.00** |
| | S×T | 1 | 3.77 | 0.05 | 0.83 |
| | S×O | 1 | 1.81 | 0.02 | 0.88 |
| | T×O | 1 | 166.41 | 2.16 | 0.15 |
| | S×T×O | 1 | 67.98 | 0.88 | 0.35 |
| | Residual | 40 | 77.20 | | |
| $\alpha_{ETR}$ | Season (S) | 1 | 0.243 | 214.64 | **0.00** |
| | Time (T) | 1 | 0.000 | 0.40 | 0.53 |
| | Origen algae (O) | 1 | 0.005 | 4.00 | **0.05** |
| | S×T | 1 | 0.001 | 0.64 | 0.43 |
| | S×O | 1 | 0.018 | 15.65 | **0.00** |
| | T×O | 1 | 0.001 | 0.45 | 0.50 |
| | S×T×O | 1 | 0.001 | 0.70 | 0.41 |
| | Residual | 40 | 0.001 | | |
| Ek | Season (S) | 1 | 89766.27 | 93.58 | **0.00** |
| | Time (T) | 1 | 697.71 | 0.73 | 0.40 |
| | Origen algae (O) | 1 | 9229.51 | 9.62 | **0.00** |
| | S×T | 1 | 345.94 | 0.36 | 0.55 |
| | S×O | 1 | 4915.13 | 5.12 | **0.03** |
| | T×O | 1 | 603.87 | 0.63 | 0.43 |
| | S×T×O | 1 | 284.56 | 0.30 | 0.59 |
| | Residual | 40 | 959.26 | | |
| $NPQ_{max}$ | Season (S) | 1 | 7.71 | 16.48 | **0.00** |
| | Time (T) | 1 | 2.66 | 5.68 | **0.02** |
| | Origen algae (O) | 1 | 1.78 | 3.82 | 0.06 |
| | S×T | 1 | 0.37 | 0.79 | 0.38 |
| | S×O | 1 | 0.58 | 1.23 | 0.27 |
| | T×O | 1 | 2.28 | 4.88 | **0.03** |
| | S×T×O | 1 | 0.80 | 1.70 | 0.20 |
| | Residual | 40 | 0.47 | | |

Table 1. – Maximum quantum yield ($F_v/F_m$), electron transport rate ($ETR_{max}$) expressed as μmol electrons $m^{-2}$ $s^{-1}$, photosynthetic efficiency ($\alpha_{ETR}$) as the initial slope of ETR versus irradiance rapid light curves (RLCs) and maximum non-photochemical quenching ($NPQ_{max}$) in winter (February) and summer (July) *C. tamariscifolia* collected from rockpools and rocky shores (field algae). Data are expressed as mean±standard deviation of n=6. Different letters indicate significant differences between period of times or collection microhabitat for each variable.

| | Winter | | Summer | |
|---|---|---|---|---|
| | Rockpools | Rocky shores | Rockpools | Rocky shores |
| $F_v/F_m$ | 0.75±0.01$^{b}$ | 0.72±0.01$^{a}$ | 0.70±0.01$^{a}$ | 0.70±0.01$^{a}$ |
| $ETR_{max}$ | 58.9±4.4$^{a}$ | 88.8±7.9$^{b}$ | 89.4±1.1$^{b}$ | 98.37±4.6$^{b}$ |
| $\alpha_{ETR}$ | 0.17±0.02$^{a}$ | 0.26±0.01$^{b}$ | 0.44±0.03$^{c}$ | 0.43±0.01$^{c}$ |
| $NPQ_{max}$ | 1.06±0.21$^{a}$ | 1.41±0.41$^{a}$ | 3.41±1.11$^{b}$ | 1.99±0.31$^{ab}$ |

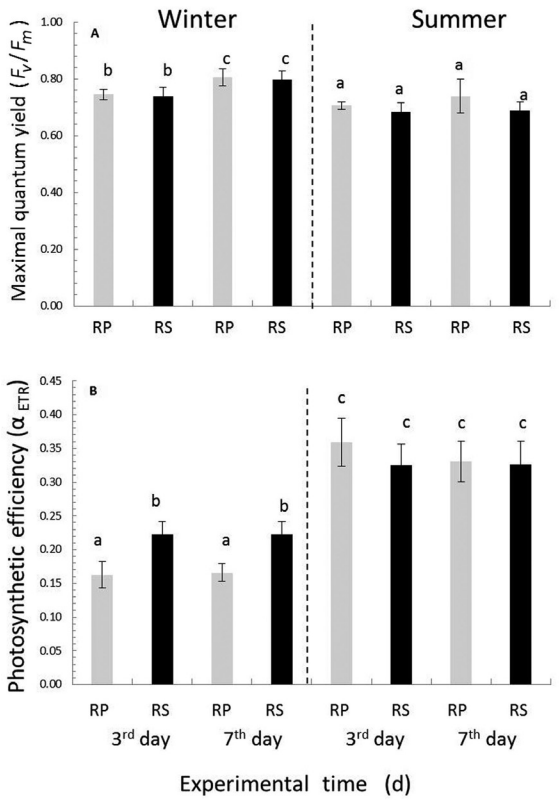


Fig. 3. – Maximum quantum yield ($F_v/F_m$) (A) and photosynthetic efficiency ($\alpha_{ETR}$) (B) in *C. tamariscifolia* during the experimental period (3rd and 7th day) in algae incubated in 1.5-L cylindrical vessels. The algae were collected in winter (February) and summer (July) and from rockpools (RP) and rocky shores (RS). Data are expressed as mean±standard deviation of n=6 and lower-case letters denote significant differences after SNK test.

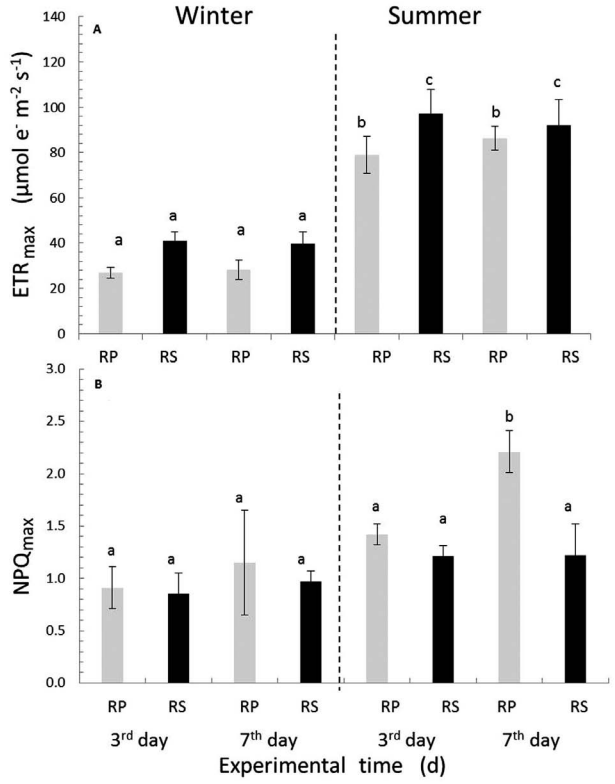


Fig. 4. – Maximum electron transport rate ($ETR_{max}$) (A) expressed as µmol electrons $m^{-2}$ $s^{-1}$ and maximum non-photochemical quenching ($NPQ_{max}$) (B) in *C. tamariscifolia* during the experimental period (3rd and 7th days) in algae incubated in 1.5-L cylindrical vessels. The algae were collected in winter (February) and summer (July) from rockpools (RP) and rocky shores (RS). Data are expressed as mean±standard deviation of n=6 and lower-case letters denote significant differences after SNK test.

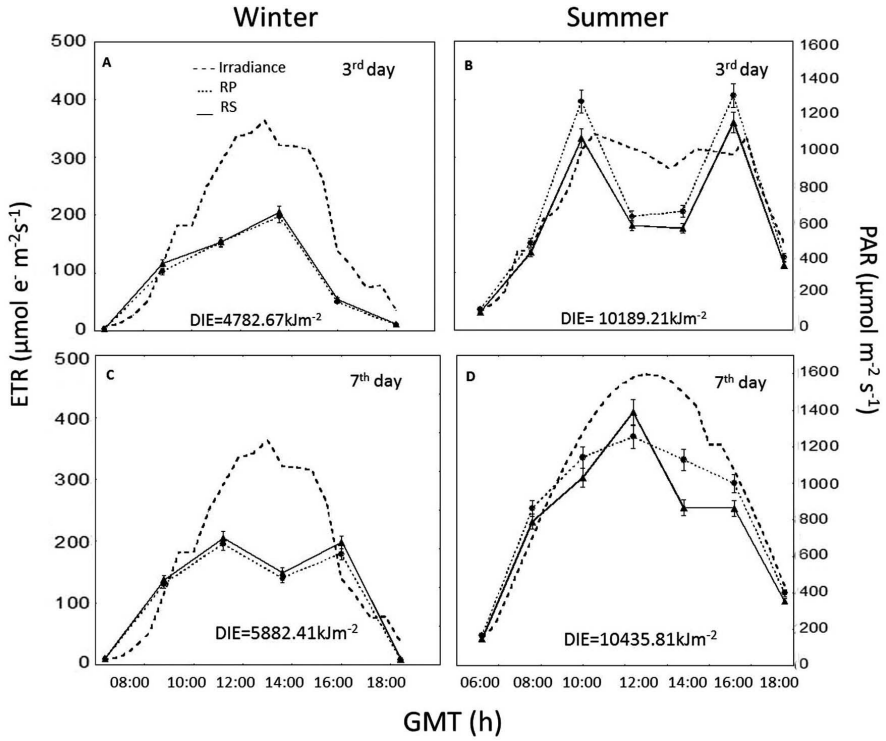


Fig. 5. – Daily cycle of in situ ETR expressed as µmol electrons $m^{-2}$ $s^{-1}$ in *C. tamariscifolia* during the experimental period (3rd and 7th days) in algae incubated in 1.5-L cylindrical vessels. The algae were collected in winter (February) (A, C) and summer (July) (B, D) and from rockpools (RP) (dotted lines) and rocky shores (solid lines) (RS). The daily irradiance of PAR expressed as µmol photons $m^{-2}$ $s^{-1}$ (straight lines). The average daily integrated irradiance (DIE) expressed as kJ $m^{-2}$ during the experimental period is indicated.

therefore 20 seconds was selected as most common time used in the literature.

$F_v/F_m$ was significantly ($p<0.05$) higher in winter than in summer both just after alga harvesting (Table 1) and after incubation for 3 and 7 days (Table 2 and Fig. 3A). $F_v/F_m$ in *C. tamariscifolia* showed a significant interaction between season and time. $F_v/F_m$ increased during the experimental time only in winter whereas, in summer a significant decrease was observed (Table 2 and Fig. 3A). The collection microhabitat (RS or RP) did not affect the values of $F_v/F_m$.

$ETR_{max}$ ($F_{1,8}$= 15.6, $p<0.05$) and $\alpha_{ETR}$ ($F_{1,8}$=11.7, $p<0.05$) was higher in thalli collected in summer than in winter (Table 1). $ETR_{max}$ and $\alpha_{ETR}$ showed a significant interaction between season and time. In winter, $\alpha_{ETR}$ (Table 2 and Fig. 3B) increased during the experimental time, whereas in summer $\alpha_{ETR}$ remained constant. $ETR_{max}$ in the experimental period was higher in thalli collected from RS than from RP, in thalli collected in summer (Table 2 and Fig. 4A); whereas, $\alpha_{ETR}$ showed the same pattern only in winter (Table 2 and Fig. 3B).

$NPQ_{max}$ was higher in field algae collected in summer than in winter and no significant differences between thalli from RP and RS were found (Table 1). $NPQ_{max}$ showed a significant interaction between time and origin of the algae. However, during the incubation period it was higher only in thalli collected from RP in summer, at the end of the experimental period (Table 2 and Fig 4B).

In situ ETR in outdoor experiments showed a daily pattern in both winter (Fig. 5A, C) and summer (Fig. 5B, D). In winter, the irradiance around noon was about 900 μmol $m^{-2}$ $s^{-1}$, whereas in summer it was 1600 μmol $m^{-2}$ $s^{-1}$ (Fig. 5). Though the daily integrated irradiance was about two times lower in winter than in summer, the ETR decreased on the 7th day in the first season. However, in summer the decrease in ETR occurred at the 3rd but not at the 7th day. The decrease in ETR in the experimental period in winter was higher in thalli collected from RP than from RS (Fig. 5A, C); whereas in summer (Fig. 5B, D) it was higher in thalli collected from RS. The period of decrease of ETR was 4 hours in winter, whereas in summer it was 6 hours. However, in both seasons, the ETR reached similar values.

Total internal N content was higher and C:N ratio lower in thalli collected in winter than in summer. ANOVA results showed a significant interaction between season and origin of the algae (Table 3 and 4). After 3 and 7 days of incubation, winter-grown thalli maintained higher levels of N and consequently lower levels of C:N than summer-grown ones (Table 3 and 4).

In summer Chl *a* and Chl $c_1+c_2$ concentrations were 1.6±0.4 mg $g^{-1}$ DW and 0.28±0.04 mg $g^{-1}$ DW, in field-collected algae, respectively, whereas, in winter they were 1.2±0.2 mg $g^{-1}$ DW and 0.17±0.04 mg $g^{-1}$ DW. After 3 and 7 days of incubation, Chl *a* showed a significant interaction between season and origin of the algae, and between time and origin of the algae (Table 3 and 5). Chl *c*1+*c*2 showed a significant interaction between season and origin of the algae (table 3 and 5). A higher content of Chl *c*1+*c*2 was found in thalli in winter.

Table 3. – ANOVA results, testing for the effect of Season, Time and Origin of algae (RP; RS) on the total internal nitrogen, C:N ratio, photosynthetic pigments (Chl *a* and Chl $c_1+c_2$), phenolic compounds, phenolic compounds released and $EC_{50}$ of *Cystoseira tamariscifolia*. Significant differences at $\alpha<0.05$ are shown in bold.

| | | df | MS | F | p |
|---|---|---|---|---|---|
| Nitrogen | Season (S) | 1 | 408.80 | 110.98 | **0.00** |
| | Time (T) | 1 | 3.49 | 0.95 | 0.34 |
| | Origen algae (O) | 1 | 8.09 | 2.19 | 0.15 |
| | S×T | 1 | 2.72 | 0.74 | 0.40 |
| | S×O | 1 | 29.61 | 8.04 | **0.01** |
| | T×O | 1 | 2.80 | 0.76 | 0.39 |
| | S×T×O | 1 | 0.34 | 0.09 | 0.76 |
| | Residual | 40 | 3.68 | | |
| Ratio C:N | Season (S) | 1 | 205.10 | 79.14 | **0.00** |
| | Time (T) | 1 | 0.98 | 0.38 | 0.54 |
| | Origen algae (O) | 1 | 11.84 | 4.57 | **0.04** |
| | S×T | 1 | 0.21 | 0.08 | 0.78 |
| | S×O | 1 | 15.32 | 5.91 | **0.02** |
| | T×O | 1 | 0.05 | 0.02 | 0.89 |
| | S×T×O | 1 | 0.47 | 0.18 | 0.67 |
| | Residual | 40 | 2.59 | | |
| Chl *a* | Season (S) | 1 | 0.88 | 7.37 | **0.01** |
| | Time (T) | 1 | 0.02 | 0.19 | 0.67 |
| | Origen algae (O) | 1 | 0.15 | 1.31 | 0.26 |
| | S×T | 1 | 0.01 | 0.01 | 0.93 |
| | S×O | 1 | 0.64 | 5.38 | **0.03** |
| | T×O | 1 | 0.66 | 5.55 | **0.02** |
| | S×T×O | 1 | 0.01 | 0.11 | 0.74 |
| | Residual | 40 | 0.12 | | |
| Chl $c_1+c_2$ | Season (S) | 1 | 0.065 | 35.67 | **0.00** |
| | Time (T) | 1 | 0.000 | 0.00 | 0.97 |
| | Origen algae (O) | 1 | 0.005 | 2.73 | 0.11 |
| | S×T | 1 | 0.000 | 0.26 | 0.62 |
| | S×O | 1 | 0.017 | 9.02 | **0.00** |
| | T×O | 1 | 0.004 | 2.31 | 0.14 |
| | S×T×O | 1 | 0.001 | 0.60 | 0.44 |
| | Residual | 40 | 0.002 | | |
| Phenolic compounds | Season (S) | 1 | 1226.40 | 63.22 | **0.00** |
| | Time (T) | 1 | 23.49 | 1.21 | 0.28 |
| | Origen algae (O) | 1 | 220.94 | 11.39 | **0.00** |
| | S×T | 1 | 129.15 | 6.66 | **0.01** |
| | S×O | 1 | 637.88 | 32.88 | **0.00** |
| | T×O | 1 | 175.16 | 9.03 | **0.00** |
| | S×T×O | 1 | 8.04 | 0.41 | 0.52 |
| | Residual | 40 | 19.40 | | |
| Release of phenolic compounds | Season (S) | 1 | 0.07 | 1.55 | 0.22 |
| | Time (T) | 1 | 0.67 | 14.61 | **0.00** |
| | Origen algae (O) | 1 | 0.63 | 13.70 | **0.00** |
| | S×T | 1 | 0.25 | 5.53 | **0.02** |
| | S×O | 1 | 0.01 | 0.09 | 0.76 |
| | T×O | 1 | 0.09 | 2.00 | 0.16 |
| | S×T×O | 1 | 0.10 | 2.08 | 0.16 |
| | Residual | 40 | 0.05 | | |
| $EC_{50}$ | Season (S) | 1 | 3.48 | 202.76 | **0.00** |
| | Time (T) | 1 | 0.35 | 20.46 | **0.00** |
| | Origen algae (O) | 1 | 0.36 | 21.43 | **0.00** |
| | S×T | 1 | 0.03 | 1.94 | 0.17 |
| | S×O | 1 | 0.48 | 28.26 | **0.00** |
| | T×O | 1 | 0.11 | 6.50 | **0.01** |
| | S×T×O | 1 | 0.01 | 0.06 | 0.81 |
| | Residual | 40 | 0.02 | | |

The phenolic compound content was higher ($F_{1,8}$=5.8, $p<0.05$) in field-collected algae in winter than in summer. After the experimental periods, ANOVA results showed significant interactions between season and time, between season and origin of the algae and between time and origin of the algae (Table 3 and 6). In winter, phenol concentration increased from 25 to 41 mg $g^{-1}$ DW in RP algae from the 3rd to 7th d incubation but decreased from 41 to 27 mg $g^{-1}$ DW in RS algae. In summer, phenolic compounds did not change in RP

Table 4. – Concentration of internal N expressed as mg $g^{-1}$ DW and C:N ratio in *C. tamariscifolia* collected in winter (February) and summer (July) from rocky shores (RS) and rockpools (RP) from field material (field algae) and after 3 and 7 d incubation in 1.5-L UV-transparent cylindrical vessels under solar radiation (incubated algae). Data are expressed as mean±standard deviation of n=6 and lower-case letters denote significant differences after SNK test.

| | | Winter | | Summer | |
|---|---|---|---|---|---|
| | | N | C:N | N | C:N |
| Field algae | RP | 21.0±0.5 [b] | 13.9±0.4 [a] | 18.2±1.3 [a] | 15.7±1.0 [b] |
| | RS | 22.2±0.5 [b] | 13.3±0.7 [a] | 18.6±1.2 [a] | 14.8±1.1 [b] |
| Incubated algae 3rd day | RP | 22.1±1.6 [c] | 12.5±1.2 | 15.4±2.6 [a] | 17.8±2.3 |
| | RS | 22.0±1.7 [c] | 12.8±0.8 | 18.2±1.9 [b] | 15.5±1.7 |
| Incubated algae 7th day | RP | 23.8±2.3 [c] | 12.2±1.2 | 15.8±1.6 [a] | 17.4±2.1 |
| | RS | 22.4±1.2 [c] | 12.8±1.5 | 17.8±2.0 [b] | 15.6±1.9 |

Table 5. – Concentration of internal Chl *a* and Chl $c_1$+$c_2$ expressed as mg $g^{-1}$ DW in *C. tamariscifolia* collected in winter (February) and summer (July) from rocky shores (RS) and rockpools (RP) from field material (field algae) and after 3 and 7 d incubation in 1.5-L UV-transparent cylindrical vessels under solar radiation (incubated algae). Data are expressed as mean±standard deviation of n=6 and lower-case letters denote significant differences after SNK test.

| | | Winter | | Summer | |
|---|---|---|---|---|---|
| | | Chl *a* | Chl $c_1$+$c_2$ | Chl *a* | Chl $c_1$+$c_2$ |
| Field algae | RP | 1.31±0.28 [ab] | 0.19±0.05 [ab] | 2.01±0.55 [b] | 0.23±0.09 [b] |
| | RS | 1.09±0.11 [a] | 0.13±0.07 [a] | 1.70±0.26 [ab] | 0.19±0.02 [ab] |
| Incubated algae 3rd day | RP | 1.53±0.31 | 0.22±0.04 [c] | 1.04±0.30 | 0.13±0.03 [a] |
| | RS | 1.68±0.20 | 0.23±0.02 [c] | 1.60±0.42 | 0.19±0.04 [b] |
| Incubated algae 7th day | RP | 1.74±0.26 | 0.26±0.05 [c] | 1.21±0.41 | 0.13±0.05 [a] |
| | RS | 1.36±0.37 | 0.21±0.02 [c] | 1.36±0.44 | 0.18±0.07 [b] |

Table 6. – Content of phenolic compounds (PC) expressed as mg $g^{-1}$ DW and antioxidant activity as $EC_{50}$ (mg DW $mL^{-1}$, DPPH method) in *C. tamariscifolia* collected in winter (February) and summer (July) from rocky shores (RS) and rockpools (RP) from field material (field algae) and after 3 and 7 d incubation in 1.5-L cylindrical vessels under solar radiation (incubated algae). Data are expressed as mean±standard deviation of n=6 and lower-case letters denote significant differences after SNK test.

| | | Winter | | Summer | |
|---|---|---|---|---|---|
| | | PC | $EC_{50}$ | PC | $EC_{50}$ |
| Field algae | RP | 36.94±5.09 [b] | 1.10±0.14[c] | 31.72±3.60 [a] | 0.81±0.32 [b] |
| | RS | 47.12±6.26 [b] | 0.50±0.07 [a] | 32.17±2.46 [a] | 0.40±0.05 [a] |
| Incubated algae 3rd day | RP | 25.73±2.10 [a] | 1.30±0.20 [c] | 27.01±4.78 [a] | 0.60±0.11 [a] |
| | RS | 41.95±5.16 [b] | 0.82±0.11 [b] | 27.01±5.21 [a] | 0.54±0.07 [a] |
| Incubated algae 7th day | RP | 35.04±2.03 [b] | 1.42±0.14[d] | 28.13±2.73 [a] | 0.64±0.05 [a] |
| | RS | 41.99±3.02 [b] | 1.15±0.09 [c] | 22.12±2.75 [a] | 0.75±0.08 [a] |

(27-28 mg $g^{-1}$ DW) and RS algae (27 to 22 mg $g^{-1}$ DW) (Table 3 and 6).

The release of polyphenols expressed as mg $g^{-1}$ DW $d^{-1}$ after the experimental period showed a significant interaction between season and time. PR, after 3 days of incubation was similar in thalli collected from RP in both seasonal periods (Table 3 and 7), whereas after 7 days the release was higher in summer than in winter, particularly in thalli collected from RS. The release expressed as percentage of the internal content was clearly higher after 7 days of incubation in summer- than in winter-collected *C. tamariscifolia*. After 7 days, the percentage of release was 3 and 5 times higher in summer-collected RS and RP algae, respectively, than in winter-collected ones (Table 3 and 7).

Antioxidant activity estimated as the oxidation index, $EC_{50,}$ in *C. tamariscifolia* was higher in field-collected algae in summer than in winter. $EC_{50}$ showed significant interactions between season and origin of the algae and between time and origin of the algae. It remained higher in summer-collected thalli than in winter-collected thalli in the experimental period (Table 3 and 6). Meanwhile, only in winter was the antioxidant activity higher in RS-grown than in RP-grown algae during the experimental period. In winter, the antioxidant activity decreased during the experimental time, whereas in summer no significant differences during the experimental period were found.

Table 7. – Release of phenolic compounds in seawater using phloroglucinol as standard in *C. tamariscifolia* collected in winter (February) and summer (July) from rocky shores (RS) and rockpools (RP). Data are expressed as mean (SD) percentages of phenol released to total internal content (% release) after 3 and 7 d culture in 1.5-L cylindrical vessels under solar radiation, n=6 and lower-case letters denote significant differences after SNK test. PR, phenol released (mg $g^{-1}$ DW $d^{-1}$).

| | | Winter PR | Summer PR | Winter % release | Summer % release |
|---|---|---|---|---|---|
| 3rd day | RP | 0.31±0.05[a] | 0.35±0.08[a] | 3.58 | 3.85 |
| | RS | 0.73±0.20[b] | 0.55±0.23[b] | 5.22 | 6.15 |
| 7th day | RP | 0.11±0.03[a] | 0.23±0.05[a] | 1.17 | 3.63 |
| | RS | 0.17±0.04[a] | 0.47±0.13[b] | 1.64 | 8.41 |

## DISCUSSION

Photosynthetic capacity ($ETR_{max}$) and photosynthetic efficiency ($\alpha_{ETR}$) were higher in *C. tamariscifolia* collected in summer than in winter and these differences were maintained after 7 days of incubation in cylindrical vessels under full solar radiation and the temperature of each season. In summer, during the experimental period, high daily integrated irradiance of PAR (99.77 MJ $m^{-2}$) and temperature (22°C

day/17°C night) favoured photosynthetic activity in *C. tamariscifolia* compared with the winter period (54.26 MJ $m^{-2}$ and 18°C day/12°C night). These results are in agreement with its latitudinal and zonal distribution in the coastal areas (Lüning 1990, Thibaut et al. 2005). A positive correlation between the $ETR_{max}$ calculated from the RLC and the in situ $ETR_{max}$ from the daily cycles of ETRs ($r=0.89$, $p<0.001$, $n=60$) was found: the latter were always 4.5 times higher. Parameters derived from RLCs as $ETR_{max}$ or *Ek* are sensitive to diurnal fluctuations as the effective and maximum quantum yields of PSIIs (Belshe et al. 2007).

ETRs calculated in daily cycle (in situ ETR) tend to be higher than in RLCs, as was described by Longstaff et al. (2002). The ETR was also higher under solar radiation (in situ measurements) than under the halogen lamp provided by the Diving-PAM (RLC determination). This result can be explained by the different light qualities of the radiation sources, i.e., the solar radiation has a much higher blue:red light ratio than the halogen lamp of the Diving-PAM, contributing to a higher electron flow by accessory pigments (carotenoids) to chlorophyll. Brown algae showed a high photosynthetic quantum yield in blue light according to the action spectra for photosynthesis reported by Lüning and Dring (1985).

The microhabitat of collection (RS or RP), in addition to the season, affected the photosynthetic pattern in field collected *C. tamariscifolia*. Higher $ETR_{max}$ and $\alpha_{ETR}$ in RS than in RP field-collected algae was observed, but only in winter. One possible explanation for this pattern is that the emersion periods in RS algae favoured photosynthetic activity by direct incorporation of $CO_2$ from the air. *C. tamariscifolia* can grow in RP, where it is always submerged during the daily period. On the other hand, *C. tamariscifolia* growing in RS may be subjected to different cycles of desiccation and rewetting, increasing atmospheric $CO_2$ uptake and nutrient incorporation; the inorganic carbon uptake in *C. tamariscifolia* growing in rockpools depend mainly on the amount of dissolved $HCO_3^-$ and carbon concentration mechanisms (CCMs) through carbonic anhydrase (CA), with the consequent energy cost (Falkowski 1997). It has been reported that intertidal algae under moderate desiccation conditions have higher nitrogen and phosphate uptakes (Lobban and Harrison 1994, Nygard and Dring 2008), photosynthetic rates (Dring and Brown 1982, Mercado et al. 1998). The carbon incorporation under emersion is higher than that under submerged conditions due to the direct uptake of $CO_2$ (Flores-Moya et al. 1998).

Internal N content was higher in winter- than in summer-collected algae, as is expected according to nitrate content in the water (Ramírez et al. 2005). Interestingly, these differences were maintained after 7 days of incubation in cylindrical vessels in spite of the nitrate enrichment (maximum level of 50 μM). Therefore, the nutritional state seems to be more favourable in winter- than in summer-grown algae as a lower C:N in thalli collected in winter was found. Additionally, thalli collected in summer seemed not to accumulate N compounds after nitrate enrichment due to a low uptake rate. It seemed that thalli accumulate N during winter as a reservoir. This result could be related to the high amount of energy that these macroalgae demand in summer, the period in which the activation of photoprotection and acclimation mechanisms may occur (Hanelt and Figueroa 2012).

This result, in combination with the higher photosynthetic rate ($ETR_{max}$) and efficiency ($\alpha_{ETR}$) in thalli collected in summer and incubated for 7 days, suggests that *C. tamariscifolia* is not limited by N in summer and it can invest the photosynthetic energy in growth. Sales and Ballesteros (2012) reported higher growth rate in *Cystoseira crinita* from the northwestern Mediterranean in summer than in winter. This is also in accordance with the higher $ETR_{max}$ in thalli collected from RS than from RP in both seasons. Moreover, Celis-Plá et al. (2014) found higher $ETR_{max}$ in *C. tamariscifolia* collected from 0.5 m depth waters than from 2.0 m depth waters in summer after an in situ experimental period. As in this study, $ETR_{max}$ at initial time was also higher in algae of the intertidal zone during the emersion than the submersion period (Nitschke et al. 2012).

Most of the differences between season and growing sites observed in the thalli collected from the field (field algae) remains after 7 days of incubation under immersed conditions in cylindrical vessels. These results indicate a high resilience of this species. The higher decay observed after 3 d of exposure in summer-grown algae and after 7 d of exposure in winter-grown algae indicates a possible accumulative inhibitory effect in winter and high photoacclimation capacity in summer-grown algae. Some authors have also shown that the dynamic photoinhibitory response may be related to acclimation responses to UV radiation (Häder and Figueroa 1997, Figueroa et al. 1997, Flores-Moya et al. 1998, Figueroa et al. 2003).

In fact, studies on daily photoinhibition and full recovery in intertidal Mediterranean algae suggest that photoinhibition is a photoprotective mechanism against high solar radiation, as in higher plants, and that the pattern of photoinhibition and recovery is affected by accumulative dose (Figueroa and Viñegla 2001). An enhanced capacity for dynamic photoinhibition and subsequent recovery has been previously reported in macroalgae, including brown macroalgae from southern Spain (Häder et al. 1998, Flores-Moya et al. 1999). In summer-grown thalli collected from RP, the ETR decay was delayed, as was observed in the daily cycle or in situ measurements. Our results suggest that photoinhibition can be a mechanism that protects *C. tamariscifolia* against high irradiance as observed in other intertidal seaweeds (Osmond 1994, Hanelt 1996). In addition to dynamic photoinhibition, another indicator of high photoacclimation capacity is the high energy dissipation that allows species to cope with excess excitation energy, as is the case of NPQ (Klughammer and Schreiber 2008). *C. tamariscifolia* specimens collected in summer and from RS showed higher values of NPQ than those collected in winter and from RP. High values of NPQ indicate active photoprotective mechanisms, which are highly related to the xanthophyll cycle (Demmig-Adams and Adams 2006).

Phenolic content was higher in winter than in summer. This could be an indicator of a good physiological status, i.e. accumulation of secondary metabolic compounds in nutrient-replete conditions (winter) to be used in nutrient-depleted conditions (summer) (Celis-Plá et al. 2014). Abdala-Díaz et al (2006) showed both seasonal and hourly variation in phenolic compounds depending on the daily integrated irradiance (dose) or hourly irradiances, respectively. It has been described that the variability in the phenolic content could be related to environmental factors such as herbivory, light, depth, salinity, nutrients and seasonality, as well as to intrinsic ones such as age, length and kind of tissue (see Amsler and Fairhead 2006, for review). Zubia et al. (2008) described a complexity of seasonal variations suggesting a stronger correlation between phenolic contents and local environmental factors (e.g. grazing intensity in different areas of the coral reef) than between large scale factors (i.e. months, seasons, latitude). The phenolic content and the antioxidant activity have been related to algal zonation (Connan et al. 2004). In the eulittoral and intertidal zone, some algae (*Fucus spiralis, Fucus vesiculosus, Ascophyllum nodosum*) show higher phenolic content than algae growing in the low intertidal or sublittoral zone (*Fucus serratus, Bifurcaria bifurcata, Himathalia elongata* and *Laminaria digitata*) (Connan et al. 2004). Also, contents are higher in summer when irradiance is the highest, as observed in several brown macroalgae from Brittany (Connan et al. 2004) and in *C. tamariscifolia* collected in Southern Spain (Abdala-Díaz et al. 2006). In contrast, in our study *C. tamariscifolia* showed higher phenolic content in winter than in summer, probably in relation to the high winter nitrate availability in Málaga (Ramírez et al. 2005). N can enhance the accumulation of phenolic compounds in some brown algae (Pavia and Toth 2000, Celis-Plá et al. 2014) as well as in *Ulva rigida* (Cabello-Pasini et al. 2011). In contrast to internal phenolic content, the release of phenolic compounds was similar in RP in winter and in summer, and higher in RS (Table 7), whereas the percentage of release to internal content was clearly higher in summer- than winter-collected algae, i.e. after 7 days of incubation the percentage of release was about 3 or 5 times higher in algae collected in RP and RS, respectively, in summer than in winter. Phlorotannins released to seawater from the tissues react with other substances to form UV-absorbing complexes (Craigie and McLachlan 1964, Carlson and Carlson 1984, Jennings and Steinberg 1994, Dujmov et al. 1996). However, a few data are available on quantities of released phlorotannins (Toth and Pavia 2000) or on their physiological and ecological function. Swanson and Druehl (2002) reported high excretion of phenols by increasing UV radiation. Although the effect of UV radiation on release rates was not directly examined in our study, there is a positive relationship between solar incident irradiance of PAR, UVA and UVB and rate of phenol release. The release rate of polyphenols in our study was about 3-5 times lower than that observed by Jennings and Steinberg (1994) in *Eklonia radiata* (10-24 $\mu g\ g^{-1}$ DW $d^{-1}$). The release rate can be related to the light history and the species, i.e. *C. tamariscifolia* is an intertidal species subject to higher daily integrated irradiance than the subtidal species *Ecklonia radiata*. High PAR irradiances and emersion have been associated with increasing phlorotannin release rates (Ragan and Jensen 1978, Carlson and Carlson 1984). In addition, phlorotannins in macroalgae are produced and released into seawater during periods of UVA stress and they are released but under UVB i.e at concentration of >0.84 g $mL^{-1}$, they reduce the impact of UVB exposure in UV-sensitive kelp meiospores (Roleda et al. 2006, Huovinen et al. 2010). Taking into account that phlorotannins exhibit absorption maxima at 200 and 270 nm, the putative shielding capacity of phlorotannins would be more efficient in the case of DNA damage (caused mainly by UV-B wavelengths) than photosynthesis, for example, which is normally also affected by wavelengths in the UV-A region (Huovinen el at. 2010). Koivikko et al. (2005) also described exudation of phlorotannins to the surrounding water, and the rate of exudation was not affected by nutrient shortage. Karban and Baldwin (1997) reported an indirect defence of phlorotannins in algae, i.e. increased excretion of these compounds into the water when algae were grazed.

*C. tamariscifolia* showed higher antioxidant capacity in thalli collected in summer than in winter and in thalli collected from RS than from RP in spite of the lower content of internal phenols. Therefore, high antioxidant activity is produced in algae submitted to high solar irradiances and low internal N content. Since the internal polyphenol content is lower in summer than in winter, we suggest that the antioxidant activity in summer could be related to other internal substances such as carotenoids.

## CONCLUSIONS

Photoacclimation capacity of *C. tamariscifolia* was higher in thalli collected in summer than in winter and in thalli from RS than from RP, i.e. the algae are less vulnerable to increased solar exposure when subject to more stressful conditions (e.g. high solar irradiance and low nitrate level). In thalli collected in summer from RS, photosynthetic activity was higher and photoinhibition lower after 7 days of incubation than in thalli collected in winter from rockpools. This higher acclimation capacity could be explained by: (1) high dynamic photoinhibition, as is shown during daily cycles, i.e. fast and high increase of $ETR_{max}$ and $F_v/F_m$ in the afternoon (high recovery); (2) high $NPQ_{max}$, indicating an efficient energy dissipation and high photoprotection capacity (Celis-Plá et al. 2014); and (3) high antioxidant activity (low $EC_{50}$), related not to internal phenolic compounds but probably to other antioxidant substances such as carotenoids. A high acclimation capacity to increased UVB radiation of *C. tamariscifolia* has recently been shown based on the accumulation of UV screen substances, high release rates of polyphenols and high antioxidant activity (Figueroa et al. 2014).

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