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RESEARCH ARTICLE

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Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseira tamariscifolia* (Phaeophyta)

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Abstract Effects of solar radiation on phenolic compound concentrations and photosynthetic activity, estimated as in vivo chlorophyll fluorescence, in the brown alga *Cystoseira tamariscifolia* (Hudson) Papenfuss were analyzed in southern Spain from June 1988 to July 2000. Annual and diurnal variations of optimum quantum yield were negatively correlated with incident irradiance. Optimum quantum yield decreased as irradiance increased at noon, and yield values recovered in the afternoon suggesting a dynamic photoinhibition. The annual and daily fluctuations of phenolic compound concentration in the tissue of *C. tamariscifolia* showed contrasting patterns. There was an annual cycle of phenolic compound concentration in the apical thallus, which was positively correlated with incident irradiance. The increase in phenolic compounds, however, was twofold greater in the first half of the year than the decrease during the second half of the year. In contrast to the annual cycle, there appeared to be a negative correlation between phenolic compound concentration and irradiance in the summer months while no specific relationship was observed in the fall–winter months. Loss of phenolic compounds from the tissue to the surrounding water was increased as irradiation dosage increased. This suggests that the decrease of phenolic compounds during the diurnal cycle might be regulated by the exudation of these compounds at high irradiances in the field. Collectively, our results suggest that, like dynamic photoinhibition, the rapid synthesis and turnover time of phenolic compounds in the tissue of *C. tamariscifolia* might serve as photoprotective mechanisms against high irradiances.

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Introduction

Macroalgae in temperate regions, such as the Mediterranean coast of Spain, are exposed to high doses of ultraviolet (UV) and photosynthetically active radiation (PAR) (Häder and Figueroa 1997). The high irradiance and the transparency of shallow waters in this region suggest that macroalgae might have developed more efficient photoprotective mechanisms to tolerate light stress than species from other biogeographical regions. Studies have revealed, for example, the occurrence of dynamic photoinhibition of photosynthesis under high solar radiation, which depends on daily changes in irradiance, vertical light attenuation, or to a combination of both (Häder and Figueroa 1997). Dynamic photoinhibition is a reversible process which inactivates photosystem II (PSII) when incident light exceeds the electron transport capacity. While daily variations of optimum quantum yield are regulated by fluctuations in irradiance (Figueroa et al. 1997), less is known about annual fluctuations in optimum quantum yield.

In intertidal species, besides dynamic photoinhibition, photoprotection represents an efficient physiological adaptation to tolerate deleterious irradiances when low tides coincide with high solar radiation dosages. Phlorotannins are halogenated polymers of phloroglucinol (1,3,5-trihydroxybenzene) which frequently accumulate in large quantities in brown seaweeds (Ragan and Glombitza 1986). These phlorotannins have been linked to a variety of functions in phaeophytes including deterring grazers from feeding on the algae (Van Alstyne and Paul 1990), preventing bacterial infections (Conover and Sieburth 1964), serving as allelopatic agents (Conover and Sieburth 1966), and protecting algae from high PAR and UV damage (Pavia et al. 1997).

Metabolic compounds such as pigments, proteins, carbohydrates, and fiber in marine macrophytes show

seasonal fluctuations. These long-term fluctuations are regulated by seasonal fluctuations of irradiance, temperature, salinity, etc. (Sieburth and Jensen 1969; Cabello et al. 2004). Similarly, phenolic compounds in the tissue of seaweeds have also been shown to vary throughout the year (Ragan and Jensen 1978). Seasonal variations in phenolic compounds are species specific, but maximum values are generally observed during the summer and low values during fall and winter (Connan 2004). In addition to seasonal variation, Connan (2004) observed short-term (daily) variation in phenolic compounds in *Pelvetia canaliculata*, *Acscophyllum nodosum*, and *Bifurcaria bifurcata*. These daily variations appear to be positively correlated with air temperature when seaweeds are exposed at low tides. High irradiance levels have also been shown to decrease phenolic compounds in the tissue of *Laminaria digitata* and *L. agardhii* in short-term laboratory experiments (Sieburth and Jensen 1969). It is unknown, however, if the daily or annual variation in phenolic compound concentration in the tissue of seaweeds is regulated by irradiance levels in the field.

Brown algae can exude phenolic compounds causing a yellow color of surrounding seawater or culture media, and these compounds have been suggested as contributors to the 'gelbstoff' in seawater (Craigie and McLachlan 1964; Ragan and Jensen 1978). These compounds have been reported to be biologically active constituents of the primary cell wall (Wallace and Fry 1994). Phenolic compounds are thought to have various functions in the cell walls of plants, including the inhibition of enzymatic degradation of hemicellulose. Other studies suggest that these compounds might serve as sites for the synthesis of lignin and as UV receptors involved in the control of phototropism in plant tissue (Wallace and Fry 1994). The regulatory process of exudation of these phenolic compounds by seaweeds, however, has not been clearly established.

The multiple functions of phenolic compounds in plants imply that phlorotannin production in algae is probably determined by interactions among several biotic and abiotic factors (Targett and Arnold 1998). The phlorotannin content of brown seaweeds varies spatially and temporally due to environmental factors such as nitrogen availability (Ragan and Glombitza 1986; Arnold et al. 1995), solar and artificial UV radiation (Pavia et al. 1997; Pavia and Brock 2000), and grazing by herbivores (Van Alstyne 1988). There is little information, however, about the photoprotective characteristics of these phenolic substances and about their metabolic regulation (Targett and Arnold 1998). Consequently, the objective of this study was to evaluate the effect of high solar radiation on annual and diurnal cycles of optimum quantum yield and phenolic compound concentration in the tissue of the intertidal phaeophyte *Cystoseira tamariscifolia*.

Materials and methods

Thalli of upper intertidal populations of *C. tamariscifolia* (Hudson) Papenfuss were collected from the intertidal in the Natural Park El Cabo de Gata—Níjar (36°52′N, 2°12′W), Almería, southern Spain, from June 1998 to July 2000.

Incident solar PAR at the sampling site was measured using an ELDONET radiometer (Real Time Computer, Möhrendorf, Germany) every minute throughout the day. Daily-integrated irradiance of quantum solar energy was determined by integrating the instantaneous irradiances from dawn to dusk.

In vivo chlorophyll fluorescence of PSII was determined ($n = 10$) with a portable pulse amplitude modulated fluorometer (PAM 2000, Walz, Germany). Intrinsic fluorescence (F_o) was determined after maintaining the tissue in darkness for 15 min. A saturating actinic light pulse (9,000 µmol photon m^{-2} s^{-1}, 800 ms) was applied to obtain maximum fluorescence (F_m) in the dark adapted samples. Variable fluorescence (F_v) was determined as the difference between F_m and F_o, and optimum quantum yield (F_v/F_m) was calculated as the rate of F_v to F_m (Schreiber et al. 1986).

The phenol concentration in the tissue of *C. tamariscifolia* was evaluated by collecting apical thalli in the field (0.25 g wet weight, WW, $n = 3$) and immediately freezing them in liquid nitrogen. Samples were transported in liquid nitrogen to the laboratory and were stored at −20°C until phenolic compound concentration analyses were conducted. Samples were pulverized with a mortar and pestle in liquid nitrogen, and extracted overnight in centrifuge tubes with 2.5 ml of 80% (v/v) methanol. The mixture was centrifuged at 19,000 g for 15 min and the supernatant was collected. Total phenolic compounds were determined using phloroglucinol as a standard (Folin and Ciocalteu 1927). Phenolic concentration was expressed as mg g dry $weight^{-1}$ (DW) after determining the wet to dry ratio in the tissue (5.13).

Annual variation in optimum quantum yield ($n = 10$) was determined monthly at sunrise. In addition, daily variations of optimum quantum yield ($n = 10$) were determined in the field every 2 h in sun-exposed tissue of *C. tamariscifolia* as described above. The daily cycle of phenolic compounds was determined by collecting samples every 2 h from sunrise to sunset. Samples were frozen in liquid nitrogen and transported to the laboratory for analysis as described above.

The effect of irradiance on the accumulation of phenolic compounds was studied by incubating apical tissue (200 g) of *C. tamariscifolia* in 2-l containers with seawater. Irradiance was supplied with a 135-W low-pressure sodium lamp (Philips) at 350 and 700 µmol photon m^{-2} s^{-1}. Tissue was maintained in a cold room at 15°C with constant aeration. Water samples (1 ml) were collected from the containers approximately every

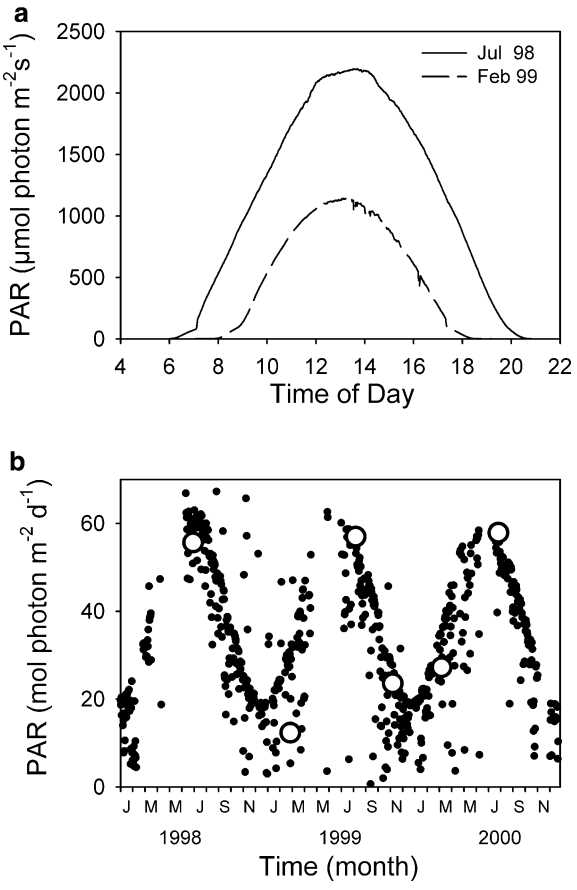

Fig. 1 **a** Daily variation of photosynthetically active radiation (PAR) in July 1998 and February 1999 at the study site; **b** Daily-integrated PAR throughout the study period. *Open circles* indicate irradiance during sampling periods

16 h and the content of phenolic compounds determined as described previously.

Significant differences of optimum quantum yield and phenolic compound concentration in the tissue of *C. tamariscifolia* as a function of irradiance was determined by a one-way ANOVA after testing for normality and homoscedasticity of the data (Sokal and Rohlf 1995). All pairwise multiple comparisons were conducted using Tukey's test. The significance of correlations between phenolic compound concentration, optimum quantum yield, and irradiance levels were tested using Pearson's product–movement correlations. Minimum significance level was established at $P < 0.05$. Best-fit linear regressions were drawn in the figures using SigmaPlot (SPSS Inc., USA) and the coefficient of determination (r^2) calculated according to Sokal and Rohlf (1995).

Results

Solar radiation

Maximal total irradiance at solar noon was approximately twofold greater in summer (approximately 2,200 µmol photon m^{-2} s^{-1}) than in winter (approximately 1,000 µmol photon m^{-2} s^{-1}) at the sampling site (Fig. 1a). Daily-integrated irradiance followed a typical sinusoidal pattern throughout the year, and was about fourfold greater in June than in December (Fig. 1b).

In vivo chlorophyll fluorescence in *C. tamariscifolia*

There was a clear seasonal fluctuation of F_v/F_m with higher values in February and November, and lower values in July (Fig. 2a). Values of F_v/F_m showed a negative correlation ($P < 0.01$, $r = -0.86$, df = 8) with increasing irradiance (Fig. 2b). Besides the annual fluctuation, there was a clear fluctuation of F_v/F_m throughout the day with lower values at solar noon, and greater values in the morning and late afternoon (Fig. 3a). In general, F_v/F_m decreased to lower levels in the summer months than in the fall–winter months, however, in all cases, values recovered completely at the end of the day. Furthermore, there was a negative correlation ($P < 0.001$, $r = -0.72$, df = 334) between irradi-

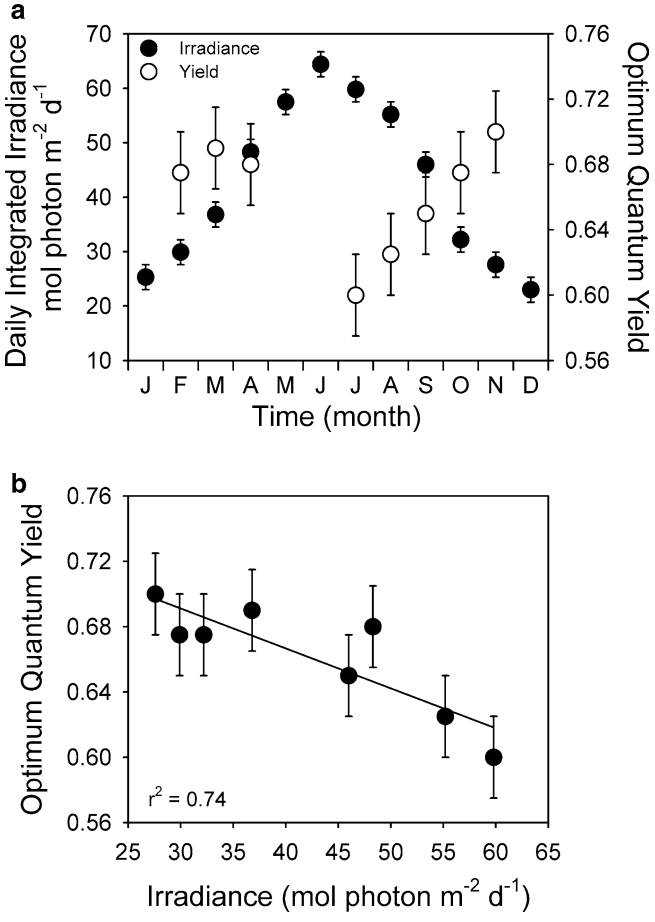

Fig. 2 *Cystoseira tamariscifolia*. **a** Annual variations of daily-integrated irradiance of PAR at the study site and optimum quantum yield (F_v/F_m) of *C. tamariscifolia* in the field; **b** Relationship of optimum quantum yield and daily-integrated irradiance of PAR

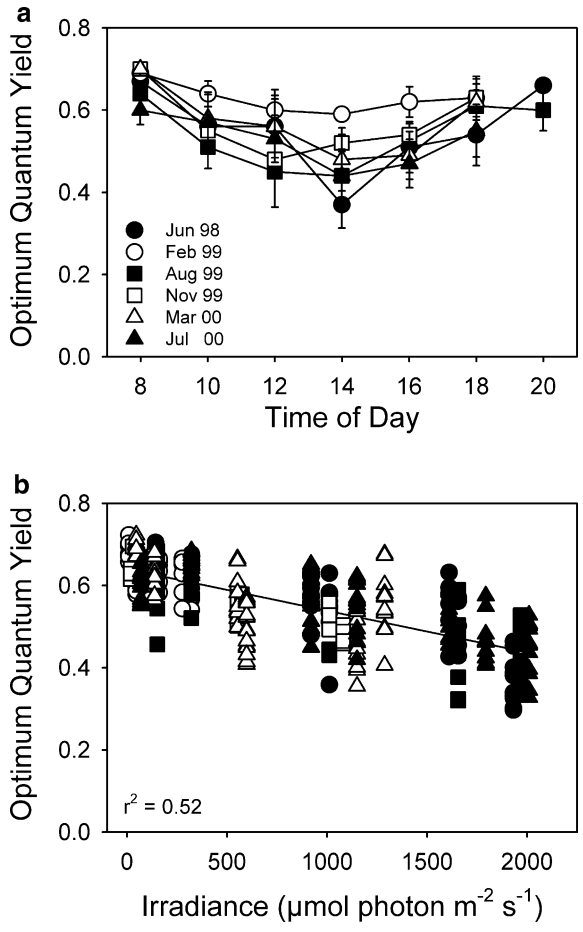

Fig. 3 *Cystoseira tamariscifolia*. **a** Daily variation of optimum quantum yield (F_v/F_m) from June 1998 to July 2000. **b** Relationship of optimum quantum yield and PAR. *Error bars*not shown were smaller than symbols

ance and F_v/F_m with greater values at low irradiances and low-yield values at high irradiances (Fig. 3b).

Phenolic compounds

There was a seasonal fluctuation of phenol concentration in the upper thallus of *C. tamariscifolia* (Fig. 4). In general, phenolic compound levels in the thallus were approximately two to fourfold greater in the summer than in the fall–winter (Fig. 4a). In general, higher phenolic compound concentrations in the apical tissue of *C. tamariscifolia* were observed in months with the greatest irradiance in the field. There was an exponential increase in phenolic compounds in the first half of the year as daily-integrated irradiance increased from February to June. In contrast, there was an exponential decrease in phenolic compounds in the second half of the year as daily-integrated irradiance decreased from June to November. The increase in phenolic compounds, however, was twofold faster from February to June than the phenolic compound decrease from June to November. The phenolic compound concentration at the end of the growth season (November) of *C. tamariscifolia* was approximately threefold greater than at their onset of growth in February.

There was a negative correlation ($P < 0.05$, $r = -0.80$, df = 8) of optimum quantum yield with phenolic compound concentration in *C. tamariscifolia* (data not shown). In general, the phenolic compound concentration in the tissue was two to threefold greater in the summer months than in the fall–winter months. Besides the annual cycles, the concentration of phenolic compounds in the tissue appeared to show diurnal cycles in the summer (Fig. 5). In June, July, and August (summer), the phenolic levels in *C. tamariscifolia* were negatively correlated ($P < 0.05$) with incident irradiance (Fig. 5a). Except for February 1999, the phenolic concentration in the tissue in the fall–winter months was not correlated with increasing irradiance (Fig. 5b).

Phenolic compound exudation

There was a positive correlation ($P < 0.05$) of phenolic compound exudation with incubation time in both the

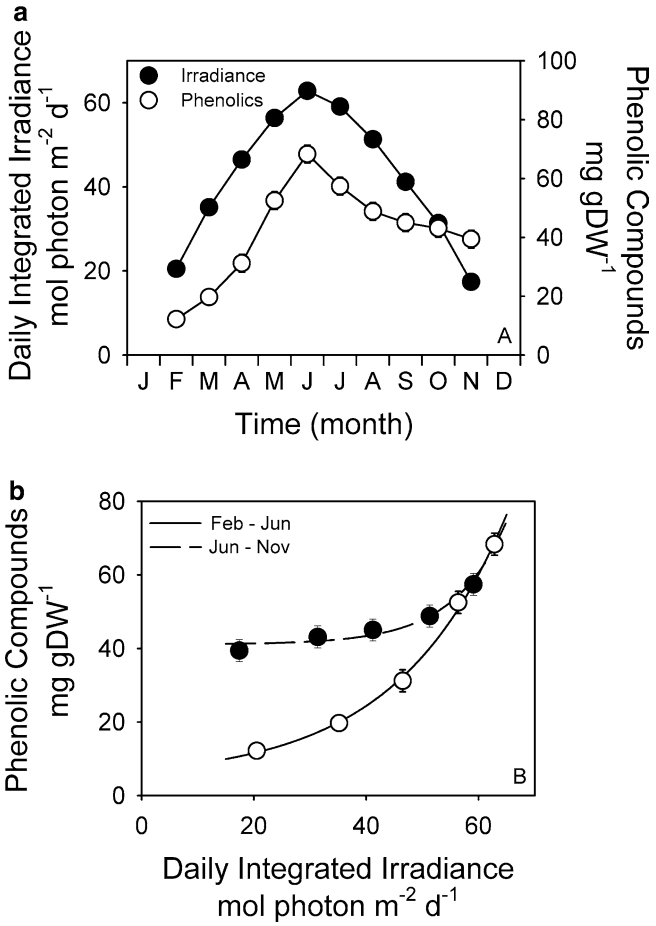

Fig. 4 *Cystoseira tamariscifolia*. **a** Annual variation of daily-integrated irradiance and phenolic compound concentration. **b** Relationship of phenolic compound concentration and daily-integrated PAR throughout the study period. *Open symbols* indicate first half of year (January–June) and *dark symbols* indicate second half of year (July–December)

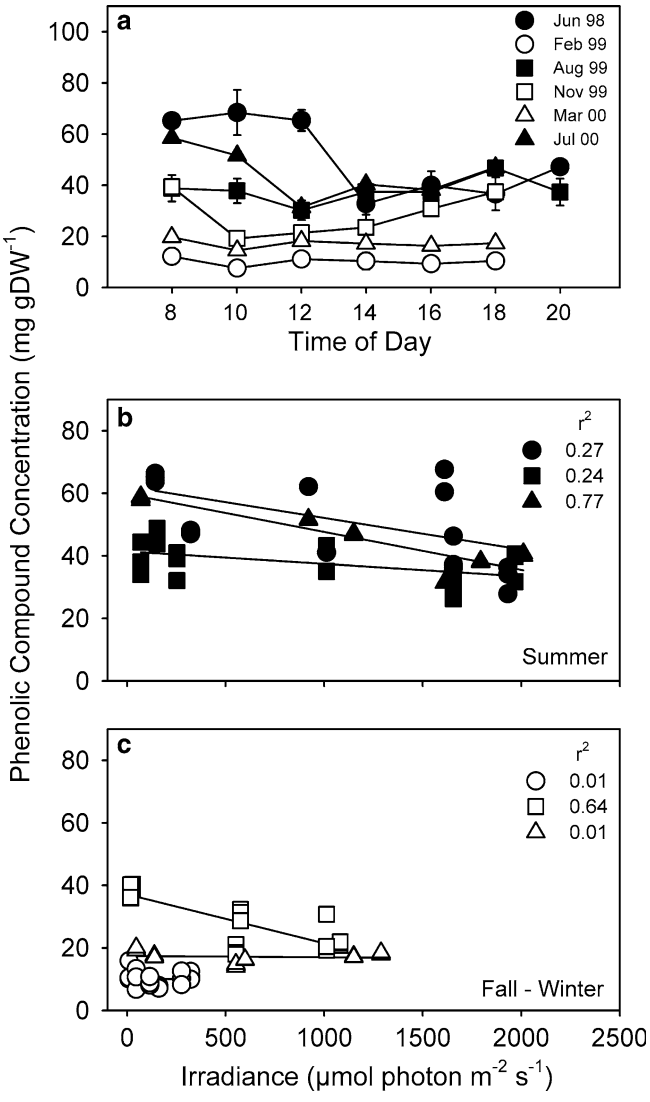

Fig. 5 *Cystoseira tamariscifolia1*. **a** Daily variation of phenolic compound tissue concentration from June 1998 to July 2000. *Filled symbols* indicate summer samples and *open symbols* indicate fall–winter samples. *Error bars* not shown were smaller than symbols. **b** Relationship of phenolic compound concentration in tissue and PAR in summer samples; **c** fall–winter samples

high and low-irradiance treatments (Fig. 6a). The slope of the exudation versus incubation period relationship, however, was fourfold greater in the high-irradiance treatment (0.78 mg l^{-1} h^{-1}) than in the low-irradiance treatment (0.19 mg l^{-1} h^{-1}). In general, the phenolic compound exudation from the tissue of *C. tamariscifolia* showed a significant correlation ($P < 0.001$, $r = 0.95$, df = 10) with increasing irradiation dosage (Fig. 6b).

Discussion

Dynamic photoinhibition has been shown to be a mechanism of acclimation and resistance to high-irradiance levels in photosynthetic organisms. In general, sun-adapted algae exhibit dynamic photoinhibition which consists of a downregulation of PSII in order to handle excess energy and to increase heat dissipation. The daily decrease and recovery of F_v/F_m in *C. tamariscifolia* observed in our study, even during the summer, suggest photoadaptation through dynamic photoinhibitory processes. An enhanced capacity for dynamic photoinhibition and subsequent recovery has been previously reported in macroalgae, including phaeophytes from southern Spain (Figueroa et al. 1997; Häder et al. 1998). Collectively, these results suggest that dynamic photoinhibition is a mechanism that protects *C. tamariscifolia* against high irradiance as observed in other seaweeds (Osmond 1994; Hanelt 1996).

Phenolic compound concentration in the tissue of *C. tamariscifolia* fluctuated from ~2% in the winter to ~8% in the summer which is consistent with the concentration observed in other species of brown seaweeds (Pavia and Toth 2000; Connan 2004). Long-term (seasonal) variations of polyphenols have been previously reported in brown algae (Ragan and Jensen 1978; Steinberg 1995; Connan 2004). Polyphenols, for exam-

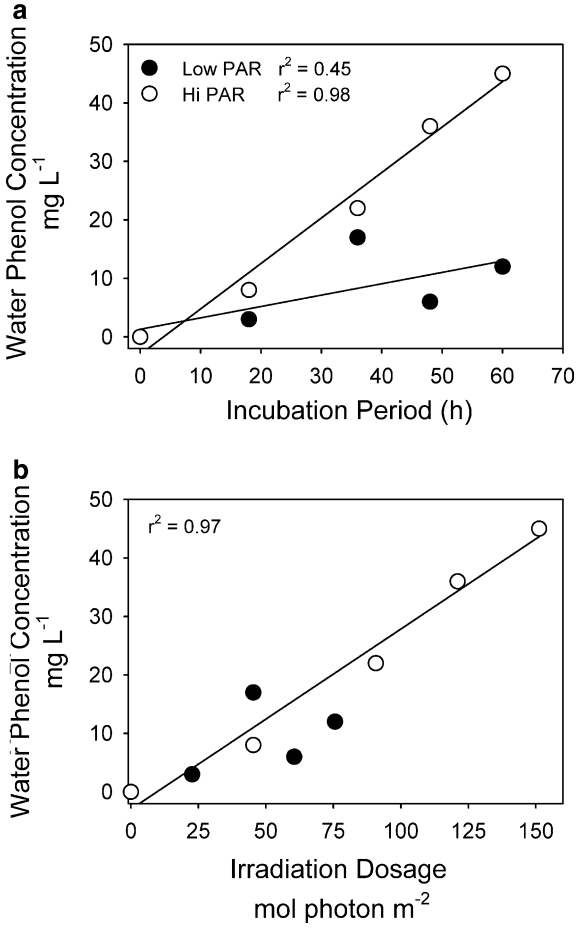

Fig. 6 *Cystoseira tamariscifolia*. **a** Exudation of phenolic compounds (expressed as milligram phenols per liter seawater) into incubation media as a function of incubation period under high and low PAR; **b** Relationship of phenolic compound exudation and irradiation dosage

ple, decreased in the summer and increased during the fall–winter in the tissue of *Ascophyllum nodosum* and *Fucus vesiculosum* in Norway. The low values appear to be correlated with a decrease in salinity during the summer. Recently, however, Connan (2004) described seasonal variation of phenolic compounds in several brown macroalgae of the Brittany coast. This seasonal variation was related to seasonal variation in antioxidant activity and related to the daily-integrated irradiance in the field. In general, the highest levels of phenolic compounds were found in summer (Connan 2004). In addition, the content of phenolic compounds and antioxidant activity decrease was related to algal zonation. Eulittoral and intertidal algae (*F. spiralis*, *F. vesiculosus*, *Ascophyllum nodosum*) growing under high daily irradiance had a higher phenolic content than algae growing in the low intertidal or sublittoral zone (*F. serratus*, *B. bifurcata*, *Himathalia elongata*, and *L. digitata*). These results are consistent with the findings in our study and collectively suggest that seasonal fluctuations of phenolic compounds in the tissue of *C. tamariscifolia* are regulated by in-situ irradiance.

The accumulation of phenolic compounds occurs more rapidly than the decrease in these compounds toward the fall–winter. The increase of phenolic compounds as summer approaches suggests that phenolic compounds might serve as a photoprotective mechanism in ecosystems with high radiation dosages such as the southern coast of Spain. This rapid increase in polyphenol compounds in the algal thallus is likely the result of the high PAR levels in the field compared to observations made in northern latitudes (i.e., Norway, Ragan and Jensen 1978). This further suggests that similar to the dynamic photoinhibition observed here, phenolic compounds might also be photoprotecting *C. tamariscifolia* against high-radiation dosages. The slower decrease in phenolic compounds, on the other hand, might be the result of the tissue degradation that occurs as winter approaches (R. Abdala, unpublished data).

Synthesis of phenolic compounds has been shown to increase in plant tissue as temperature increases (Aquino-Bolaños and Mercado-Silva 2004). Ragan and Glombitza (1986), for example, found a high correlation between surface water temperature and the content or composition of phenolic compounds in *F. vesiculosus*. Seawater temperature, however, at our study site reaches maximum values in August and September (Rodríguez-Martinez 1979), approximately 2 months after maximum levels of phenolic compounds were found in the thalli of *C. tamariscifolia*. This suggests that processes other than temperature (i.e., irradiance) might be responsible for regulating phenolic compound concentration in the thallus of *C. tamariscifolia* in southern Spain.

Similar to dynamic photoinhibition, phenolic compounds in the apical thallus could act as a photoprotection mechanism against high solar irradiance by absorbing incident photons or indirectly as a result of their antioxidant activity. Connan (2004) found a positive correlation between phenolic content and antioxidant activities in brown algae, both in long-term (seasonal) and in short-term (daily) variations. In summer, high solar irradiance can promote the synthesis of radical oxidant substances, thus the increase of phenolic compounds could act as antioxidants. Phenolic substances such as coumarins from vascular plants and trihydroxicoumarins from the green alga, *Dasycladus vermicularis*, show a high antioxidant activity against oxygen radicals (Pérez- Rodríguez et al. 2001). The regulation of trihydroxicoumarins in the tissue of seaweeds, however, is unclear as an increase of phenolic compounds was observed under high UV radiation (Pérez-Rodríguez et al. 1998; 2001) and no fluctuation of these compounds detected in juveniles and embryos of *F. gardneri* (Henry and Van Alstyne 2004). Further studies are needed to clarify the effect of UV radiation on the regulation of these phenolic compounds.

Short-term variation in phenolic compounds has also been observed in brown seaweeds. Pavia and Brock (2000) found that the phenolic content in the tissue of *A. nodosum* was regulated by tidal patterns, with greater phenolic concentrations during low tides than during immersion. Furthermore, daily variation in the phenolic content has also been found in *P. canaliculata* and *A. nodosum* on the Brittany coast (Connan 2004). This short-term variation was correlated with air temperature and irradiance in the case of *P. canaliculata* and to the tidal regime in the case of *A. nodosum* (Connan 2004). In contrast, the low intertidal brown alga *B. bifurca* showed no daily variations in either phenolic compound concentration or antioxidant activity (Connan 2004). Daily variation of phenolic compounds in *P. canaliculata* and *A. nodosum* at the end of spring fluctuated almost twofold (4.3–7.8 mg gDW^{-1}). This fluctuation was related to daily variation in antioxidant activities (Connan 2004) suggesting the existence of a very short-term variability in defense mechanisms inducted by solar irradiance. Similarly, Swanson and Druehl (2002) found significant variation of phenolic content and composition in *Macrocysitis integrifolia* with time courses of approximately 8 h, indicating relatively rapid turnover rates as observed in some Fucales (Arnold and Targett 2000). Similar to optimum quantum yield, our observations revealed fluctuations of phenolic compounds in the tissue of *C. tamariscifolia* in a short time period (i.e., hours). In contrast to the seasonal fluctuation of phenolic compounds, the diurnal phenolic compound fluctuation appears to be negatively correlated with irradiance levels, especially in the summer months. The release of phenolic compounds also appears to be coupled to higher irradiance dosages. Collectively, these results suggest that short-term variations of phenolic compounds in the tissue of *C. tamariscifolia* appear to be regulated by the exudation of these compounds under high irradiances. This is consistent with observations by Swanson and Druehl (2002) who also concluded that short-term variation in phenolic compounds in *Macrocystis integrifolia* was related to their exudation rate.

While daily fluctuations account for only 20% of the annual variations, our study shows for the first time that the concentration of polyphenols in brown algae may be linked to irradiance levels in the field which suggests that the synthesis of these compounds has a very rapid turnover time.

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