

# The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments

Fátima Fernandes<sup>1</sup> · Mariana Barbosa<sup>1</sup> · Andreia P. Oliveira<sup>1</sup> · Isabel C. Azevedo<sup>2</sup> · Isabel Sousa-Pinto<sup>2,3</sup> · Patrícia Valentão<sup>1</sup> · Paula B. Andrade<sup>1</sup>

Received: 11 March 2016 / Revised and accepted: 27 May 2016 / Published online: 7 June 2016  
© Springer Science+Business Media Dordrecht 2016

**Abstract** Macroalgae drive the biodiversity and functioning of many shallow benthic ecosystems. Besides their key role in coastal ecosystems, they are rich sources of a wide variety of unique molecules with high impact in food science, pharmaceutical industry and public health. Carotenoids and chlorophylls present in three kelp species—*Laminaria ochroleuca*, *Saccharina latissima* and *Saccorhiza polyschides*—were determined by high-performance liquid chromatography coupled to a diode array detector. The effect of different harvesting times, depths and growth conditions (wild vs. aquaculture) on pigment qualitative and quantitative profiles was assessed. Stipes, blades and whole macroalgae were studied. In spite of the considerable variability observed amongst all samples, fucoxanthin was evidently the main carotenoid. Overall, the relative contents of carotenoids were significantly higher than those of chlorophylls. In addition, the cultivation of macroalgae in an integrated multi-trophic aquaculture system appears to increase pigment levels. Altogether, the results demonstrate the complexity of the influence of species-specific and environmental factors on pigment composition and reinforce that macroalgae cultivation systems may provide an interesting approach to optimize the production of some valuable metabolites.

**Keywords** *Laminaria ochroleuca* · *Saccharina latissima* · *Saccorhiza polyschides* · Carotenoids · Chlorophylls · HPLC-DAD

## Introduction

Macroalgae comprise an abundant and heterogeneous group of marine photosynthetic organisms that generally occur in complex habitats and are often exposed to fluctuating environmental conditions. Within brown macroalgae (Ochrophyta), kelps play a key role in cold-temperate coastal ecosystems, providing shelter and serving as food for a variety of species (Steneck et al. 2002). They are also an economically important human resource with a vast array of applications in different industrial branches, such as food, textiles and pharmaceuticals. Owing to their rich polysaccharide composition and the current demand for clean, non-fossil-fuel-based energy production, kelps have been thrown into the limelight as potential sources of biofuels (i.e. bioethanol; Smale et al. 2013; Kostas et al. 2016).

Although it is true that bioprospecting of marine sources has provided some structurally unique marine products, the search for new biologically active compounds can be considered an almost unlimited field. Amongst the great chemical diversity, chlorophylls and carotenoids are the main classes of pigments found in brown algae (Bianchi et al. 1997). The photosynthetic machinery of algae usually hosts two photosystems (PS I and PS II), which are connected via the electron transport chain (Hurd et al. 2014). Chlorophyll *a* serves as a primary photoreceptor, whereas chlorophyll *c* acts as an accessory pigment to chlorophyll *a*, not only by enhancing the light-harvesting properties but also by replacing chlorophyll *a* in PS II (Hamid et al. 2015). The light-harvesting complexes of brown algae also contain carotenoids, which play important roles as accessory pigments or as structural molecules that stabilize protein

✉ Paula B. Andrade  
pandrade@ff.up.pt

<sup>1</sup> REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira no. 228, Porto 4050-313, Portugal

<sup>2</sup> Interdisciplinary Centre for Marine and Environmental Research (CIIMAR/CIMAR), Rua dos Bragas no. 289, 4050-123 Porto, Portugal

<sup>3</sup> Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

folding in the photosynthetic apparatus (Takaichi 2011). Chemically, carotenoids are classified into xanthophylls (oxygenated compounds) and carotenes (nonpolar hydrocarbons). They derive from the polymerization of isoprene units to form regular and highly conjugated  $C_{40}$  structures (tetraterpenes) via the mevalonate and 1-deoxyxylulose 5-phosphate/2-C-methylerythritol 4-phosphate pathways (Kumari et al. 2013; Zorofchian Moghadamtousi et al. 2014).

Besides their key role in oxygenic photosynthesis, both carotenoids and chlorophylls have been described as powerful antioxidants, and their beneficial effects are well documented (Stahl and Sies 2003; Lanfer-Marquez et al. 2005; Subramoniam et al. 2012; Zorofchian Moghadamtousi et al. 2014; Gammone et al. 2015). The presence or absence of certain pigments and their relative content also provide useful information regarding the possible acclimation or photoprotection responses of an organism (Schubert et al. 2006). In fact, kelp growth and survival depend enormously on their chemical repertoire to mediate interactions with continuous environmental fluctuations (e.g. light availability, water surface temperature, salinity and nutrients) and with other organisms (Stengel et al. 2011). Previous studies have shown that, in response to tidal cycles and seasonal variations, kelps are able to adjust their metabolism by optimizing their chemical composition (e.g. levels of pigments; Henley and Dunton 1995; Aguilera et al. 2002; Gerasimenko et al. 2011). Additionally, the strong morphological thallus differentiation exhibited by most of the kelps has already been shown to be translated into significant variability in photosynthetic pigments (Gómez et al. 2005; Wang et al. 2013; Schmid and Stengel 2015). Therefore, it is not surprising that pigment levels vary considerably according to light environment (Colombo-Pallota et al. 2006); however, the combined influence of several other external and species-specific factors (e.g. thallus age, metabolic activity and physiologic function) can also deeply influence the overall pigment composition (Gómez and Wiencke 1998; Stengel et al. 2011).

This work attempts to help in identifying and systematizing the effects of different parameters in algal chemical profiles, improving the chances for production of algal-derived biologically active compounds. For this, the pigment composition of three kelp species (*Laminaria ochroleuca* Bachelot de la Pylaie; *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders; and *Saccorhiza polyschides* (Lightfoot) Batters), two perennial and an annual species, respectively, collected along the Portuguese Northern Coast and from cultivation in an integrated multi-trophic aquaculture (IMTA) system, and at sea, at different depths was studied. Although *S. polyschides* is not a true kelp of the order Laminariales (being a “pseudo-kelp” of the order Tilopteridales), it is treated here as such because it serves a similar ecological function and can be the dominant canopy-forming macroalgae along large stretches of the Northeast Atlantic coastline (Smale et al. 2013). To the best of our knowledge, the results of the chemical characterization of

macroalgae are currently limited to the analysis of wild material. As cultivation becomes more widespread, there is a need to expand knowledge on this material (Schiener et al. 2015).

The main objectives of this work were then (1) to evaluate the effect of cultivation at different depths and harvest periods on the carotenoid and chlorophyll profiles of kelps; (2) to compare the pigment composition of wild and cultivated kelps; and (3) to assess the accumulation of pigments in different algal tissues. For pigment analysis, high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) was employed.

## Materials and methods

### Standards and reagents

Standards of fucoxanthin ( $\geq 95.0\%$ ),  $\beta$ -carotene ( $\geq 95.0\%$ ) and chlorophyll *a*, as well as acetone, butylated hydroxytoluene (BHT), ethanol (EtOH) and methyl *tert*-butyl ether, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of violaxanthin (95.0 %) and zeaxanthin (97.0 %) were obtained from CaroteNature (Lupsingen, Switzerland). Standards of chlorophyll  $c_2$  (99.9 %) and pheophytin *a* (90.0 %) were from LGC Standards (Manchester, NH, USA). Methanol (MeOH) and tetrahydrofuran (THF) were from Merck (Germany).

### Stock solutions

Stock solutions of fucoxanthin, violaxanthin and zeaxanthin were prepared individually in EtOH; the solution of  $\beta$ -carotene was prepared in THF and those of chlorophyll *a*, pheophytin *a* and chlorophyll  $c_2$  in acetone. BHT was added to all standard solutions (final concentration, 0.05 %), which were kept at  $-20\text{ }^\circ\text{C}$  until use.

### Biological material

The macroalgae specimens analysed in this study were obtained from different sources (Table 1). The wild specimens of *Laminaria ochroleuca*, *Saccharina latissima* and *Saccorhiza polyschides* were collected in the rocky shores of Praia Norte ( $41^\circ 41' 49''\text{N}$ ,  $8^\circ 51' 08''\text{W}$ ), Amorosa ( $41^\circ 26'\text{N}$ ,  $8^\circ 49' 22''\text{W}$ ) and São Bartolomeu do Mar ( $41^\circ 34' 24''\text{N}$ ,  $8^\circ 47' 57''\text{W}$ ) at different seasons. The specimens cultivated at sea (*S. latissima*) were grown in vertical longlines of Póvoa de Varzim ( $41^\circ 22' 21''\text{N}$ ,  $8^\circ 46' 13''\text{W}$ ). These sites are located in Northern Portugal, where the sea surface temperature (SST) ranges from around  $11\text{ }^\circ\text{C}$  in the winter to around  $22\text{ }^\circ\text{C}$  in the summer and coastal upwelling events are present, with maxima from July to September (Fiuza et al. 1982; Lemos and Pires 2004). Concerning macroalgae cultivated in vertical longlines, they

**Table 1** Details of macroalgae samples

Sample	Species	Tissue	Origin	Date of collection
Lo_W_N_Oct12	<i>L. ochroleuca</i>	Whole	Praia Norte	October 2012
Lo_B_N_Dec12	<i>L. ochroleuca</i>	Blades	Praia Norte	December 2012
Lo_S_N_Dec12	<i>L. ochroleuca</i>	Stipes	Praia Norte	December 2012
Lo_W_N_Dec12	<i>L. ochroleuca</i>	Whole	Praia Norte	December 2012
Lo_B_SBM_Jan13	<i>L. ochroleuca</i>	Blades	São Bartolomeu do Mar	January 2013
Lo_S_SBM_Jan13	<i>L. ochroleuca</i>	Stipes	São Bartolomeu do Mar	January 2013
Lo_W_SBM_Jan13	<i>L. ochroleuca</i>	Whole	São Bartolomeu do Mar	January 2013
Lo_B_SBM_Jun13	<i>L. ochroleuca</i>	Blades	São Bartolomeu do Mar	June 2013
Lo_B_Am_Apr13	<i>L. ochroleuca</i>	Blades	Amorosa	April 2013
Lo_W_IMTA_Jan13	<i>L. ochroleuca</i>	Whole	IMTA	January 2013
Lo_B_IMTA_Apr13	<i>L. ochroleuca</i>	Blades	IMTA	April 2013
Sl_B(d5)_PV_Jul12	<i>S. latissima</i>	Blades (5-m depth)	Sea cultivation, vertical longlines off Póvoa de Varzim	July 2012
Sl_B(d10)_PV_Jul12	<i>S. latissima</i>	Blades (10-m depth)	Sea cultivation, vertical longlines off Póvoa de Varzim	July 2012
Sl_B(d15)_PV_Jul12	<i>S. latissima</i>	Blades (15-m depth)	Sea cultivation, vertical longlines off Póvoa de Varzim	July 2012
Sl_W_Am_Jan13	<i>S. latissima</i>	Whole	Amorosa	January 2013
Sl_W_IMTA_Jan13	<i>S. latissima</i>	Whole	IMTA	January 2013
Sl_B_IMTA_Apr13	<i>S. latissima</i>	Blades	IMTA	April 2013
Sl_W_IMTA_Apr13	<i>S. latissima</i>	Whole	IMTA	April 2013
Sp_W_N_Jan13	<i>S. polyschides</i>	Whole	Praia Norte	January 2013
Sp_W_IMTA_Jan13	<i>S. polyschides</i>	Whole	IMTA	January 2013
Sp_B_IMTA_Apr13	<i>S. polyschides</i>	Blades	IMTA	April 2013
Sp_S_IMTA_Apr13	<i>S. polyschides</i>	Stipes	IMTA	April 2013
Sp_W_IMTA_Apr13	<i>S. polyschides</i>	Whole	IMTA	April 2013

were deployed in February and sampled in July, from 5-, 10- and 15-m depths, when SST was 15.2 °C, decreasing to 15, 14.4 and 13.8 °C at 5-, 10- and 15-m depths, respectively (unpublished data). Light intensities measured at those depths were around 780, 550 and 360  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively. Specimens cultivated in a pilot land-based installation (*L. ochroleuca*, *S. latissima* and *S. polyschides*), as in Abreu et al. (2011), were grown in outdoor tanks, tumbling in the water column, from January to April at an initial density of 2 kg  $\text{m}^{-2}$ , under water temperatures ranging from 13–14 °C in January to 13.5–16 °C in April. Light intensity at the tanks surface ranged from 500 to 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  over the cultivation period, depending on cloud cover.

After collection, macroalgae were immediately transported to the laboratory in insulated, sealed cool boxes to prevent alterations, where they were thoroughly washed with a saline solution (3.5 % NaCl) to remove epiphytes and encrusting material. Each sample corresponds to a pool of ten individuals in the same stage of development: five were studied as whole individuals and the other five split into blades and stipes in order to assess pigment content in those tissues separately. All samples were kept at  $-20$  °C prior to their lyophilisation in a Virtis SP Scientific Sentry 2.0 apparatus (Gardiner, NY, USA).

The dried material was powdered ( $<910 \mu\text{m}$ ) and kept in the dark, in a desiccator, before use.

### Extract preparation

Extracts were prepared with approximately 1 g of each dried macroalga, using 20 mL of acetone with 0.05 % BHT, under the following conditions: 15 min of sonication followed by 45 min of stirring maceration (600 rpm) at room temperature. Each sample was extracted three times. The obtained extracts were combined, filtered under vacuum and evaporated at reduced pressure (Rotavapor R-215, Büchi Labortechnik, Switzerland) until complete dryness. The dried extracts were kept at  $-20$  °C and protected from light until analysis.

### HPLC-DAD analysis

Briefly, the dried residue of each algae extract was redissolved in MeOH Lichrosolv, sonicated, filtered through a 0.45- $\mu\text{m}$  size pore membrane (Millipore) and then analysed on an analytical HPLC unit (Gilson Medical Electronics, France) according to the procedure previously described by Oliveira et al. (2015). Spectral data from all peaks were collected in the range

of 200–700 nm, and chromatograms were recorded at 450 nm. The data were processed on a Unipoint System software (Gilson Medical Electronics). The compounds were identified by comparing their retention times and UV–Vis spectra in the range of 200–700 nm with those of authentic standards injected under the same chromatographic conditions.

For quantification purposes, 20  $\mu\text{L}$  of each algal extract was analysed under the same analytical conditions. Peak purity was checked by the software contrast facilities. Pigment quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. All compounds were quantified as themselves, excepting the *cis* isomers of fucoxanthin (**2** and **3**), which were quantified as fucoxanthin (**1**), and pheophytin *a* (**7**) and chlorophyll *c* derivatives (**9** and **10**), determined as chlorophyll *a* (**6**).

### Statistical analysis

All the analyses were performed in triplicate and mean values are reported. The pigment content of the samples was compared by one-way analysis of variance (post hoc Tukey) using IBM SPSS Statistics for Windows version 23.0 (Armonk, NY, USA). Differences at  $p < 0.05$  were considered statistically significant.

## Results and discussion

### General overview of photosynthetic pigment composition

The analytical methodology employed in this work allowed the determination of six carotenoids, comprising five xanthophylls (**1–5**) and  $\beta$ -carotene (**8**), as well as of chlorophyll *a* (**6**) and of its demetalated derivative, pheophytin *a* (**7**) (Fig. 1). Compounds with chlorophyll *c*-like UV–Vis spectrum were also detected in some samples, being labelled as chlorophyll *c* derivatives (**9** and **10**) (Fig. 1).

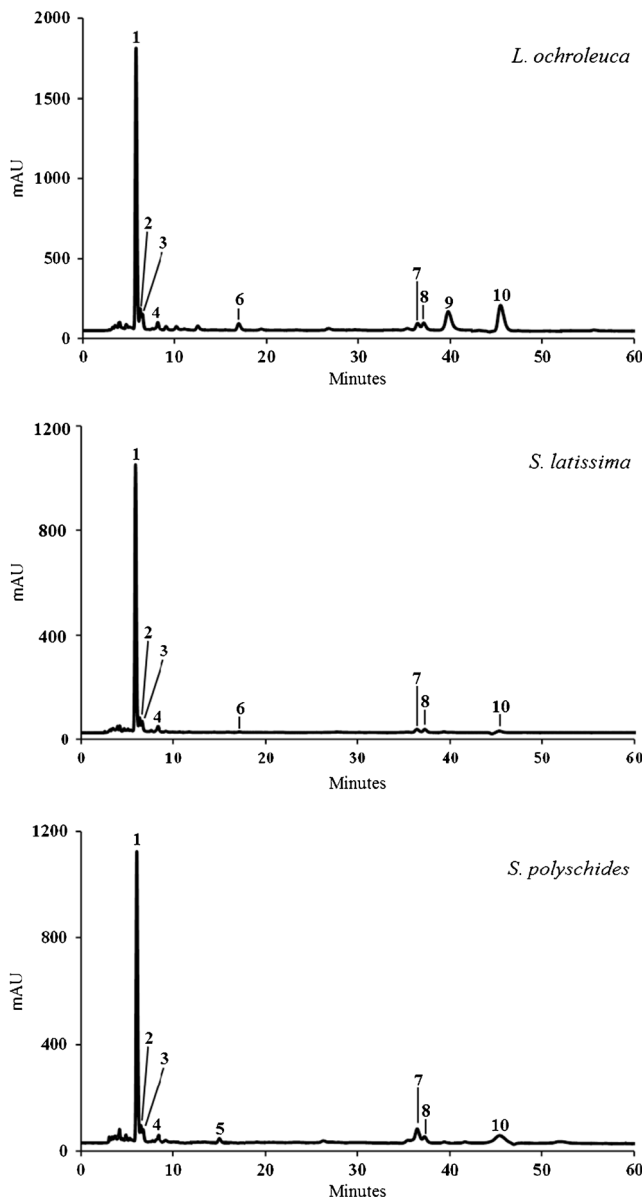
The pigment composition varied considerably amongst the analysed kelps (Table 2). However, as expected, fucoxanthin (**1**), the chemotaxonomic marker of Ochrophyta (Takaichi 2011), was found in all samples, along with its *cis* isomers (**2** and **3**). Fucoxanthin (**1**), firstly isolated from marine brown macroalgae of the genera *Fucus*, *Dictyota* and *Laminaria* (Willstätter and Page 1914), represents more than 10 % of the total carotenoids in nature (Peng et al. 2011). Besides playing a central role in macroalgae as a component of the light-harvesting complex for photosynthesis and photoprotection, this xanthophyll also displays promising effects in human health (Peng et al. 2011; Ibañez and Cifuentes 2013). Amongst the carotenoids detected in this work, fucoxanthin (**1**) was the dominant one, its relative content ranging from 4.3 up to 82.4 % of all quantified pigments.

Total pigment concentrations ranged between 34.99 and 689.21  $\text{mg kg}^{-1}$  of dry algae (Table 2). The lowest pigment levels were presented by whole-specimen samples of *S. latissima* from Amorosa beach, collected in January 2013 (Sl\_W\_Am\_Jan13), whereas samples of whole specimens of *S. polyschides* grown in the IMTA system, and collected also in January 2013 (Sp\_W\_IMTA\_Jan13), exhibited the highest amounts of photosynthetic pigments.

### Variations of the pigment composition of *L. ochroleuca*, *S. latissima* and *S. polyschides* from different harvesting periods and origins

In their natural habitats, macroalgae grow in exceptionally diverse and dynamic light climate, which is the most important and also one of the most complex abiotic factors affecting these organisms (Hurd et al. 2014). Factors other than light, such as SST to which macroalgae are subjected in the normal course of the seasons, can also influence their metabolism and, consequently, their chemical composition (Andersen et al. 2013; Martins et al. 2014; Olischläger et al. 2014; Boderkov et al. 2015). The ability to acclimate and adjust photosynthesis and growth to the rapid changes in light and temperature regimes may rely on different strategies (e.g. pigment composition plasticity), as a prerequisite for macroalgal life under seasonal changes (Andersen et al. 2013; Martins et al. 2014). One of the many outcomes of this study was the identification of significant seasonal variations in the photosynthetic pigment composition of the analysed kelps. *L. ochroleuca*, collected at Praia Norte in October 2012 (Lo\_W\_N\_Oct12) and December 2012 (Lo\_W\_N\_Dec12), exhibited considerable differences in both qualitative and quantitative pigment profiles: zeaxanthin (**5**) and  $\beta$ -carotene (**8**) were found only in the whole-specimen samples collected in October (Lo\_W\_N\_Oct12), which also presented significantly higher levels of chlorophyll *c* derivative (**10**) (Table 2). Still, *L. ochroleuca* blades from São Bartolomeu do Mar, harvested in January 2013 (Lo\_B\_SBM\_Jan13) and June 2013 (Lo\_B\_SBM\_Jun13), showed even greater differences: the last contained nearly twice the levels of pigments, amongst which carotenoids corresponded to 56.1 % of the total quantified ones (Table 2). In fact, the specimens collected in June were exposed to higher light intensity and longer photoperiod, along with increasing seawater temperatures, than the ones collected in January, as in this region of the globe June coincides with the end of spring and the beginning of summer. Overall, these results point to the occurrence of some kind of photoprotective mechanism in the algae that deflects energetic resources to pigment biosynthesis, ensuring the ecological success of the species (Goss and Jakob 2010).

Regarding kelps cultivated in IMTA, total pigment contents were generally higher (Table 2). Some qualitative differences were also noticed, although with less significance. Comparing the whole individuals of each studied macroalgae species from



**Fig. 1** HPLC-DAD carotenoid and chlorophyll profiles of acetone extracts from *L. ochroleuca* (Lo\_W\_IMTA\_Jan13), *S. latissima* (Sl\_W\_IMTA\_Apr13) and *S. polyschides* (Sp\_W\_N\_Jan13). Detection at 450 nm. (1) Fucoxanthin; (2) fucoxanthin *cis* isomer 1; (3) fucoxanthin *cis* isomer 2; (4) violaxanthin; (5) zeaxanthin; (6) chlorophyll *a*; (7) pheophytin *a*; (8)  $\beta$ -carotene; (9 and 10) chlorophyll *c* derivatives

wild natural stocks and IMTA, with identical sampling period, some observations can be highlighted. For *L. ochroleuca*, the cultivated macroalgae (Lo\_W\_IMTA\_Jan13) exhibited almost 1.4 times more pigments than the wild macroalgae from São Bartolomeu do Mar (Lo\_W\_SBM\_Jan13). In *S. latissima*, the differences were even higher. Cultivated macroalgae (Sl\_W\_IMTA\_Jan13) displayed almost 4.2 times more pigments than wild *S. latissima* collected at Amorosa (Sl\_W\_Am\_Jan13). Significantly higher pigment contents were also detected in *S. polyschides* from the IMTA system (Sp\_W\_IMTA\_Jan13): almost 4.1 times more than in the macroalgae collected at Praia

Norte (Sp\_W\_N\_Jan13). Although these results could immediately imply that algae cultivation in a pilot-scale land-based system provides higher levels of photosynthetic pigments than those from wild natural stocks, such assumption would prove to be tendentious. Most likely, the differences found for pigment levels and composition are the result of the combined influence of several ecological conditions under which a species persists in its natural habitat and also of the small-scale conditions experienced within the IMTA system (e.g. nutrient loading, light penetration and local interactions between co-cultured species; Barrington et al. 2009; Azevedo et al. 2016). Nevertheless, the use of IMTA arises as a sustainable approach for macroalgae production that allows a higher control over biomass yield, chemical composition and epiphytes by manipulating some key factors (Azevedo et al. 2016). Future fully controlled experiments are needed to elucidate the relative influences of both biotic and abiotic parameters.

**Pigment distribution within thalli**

Many macroalgae, particularly the kelps, display strong morphological thallus differentiation, which has been shown to translate into biochemical gradients (Küppers and Kremer 1978; Stengel et al. 2005; Connan et al. 2006; Schmid and Stengel, 2015).

The kelps selected for this study are economically important and edible macroalgae species, with potential to be produced sustainably through commercial aquaculture, namely *S. latissima* (Marinho et al. 2015). Therefore, the selection of thallus parts rich in valuable bioactives, such as carotenoids, may improve their nutritional value. Pigment composition of different algal thallus sections, when available, was assessed in this study. Although it has been previously reported that pigment concentrations are commonly lower in the meristematic areas of algal thalli (Küppers and Kremer 1978; Schmid and Stengel 2015), such a trend was not observed in the total pigment contents for most of the analysed algal tissues (Table 2). These contrasting results can be possibly attributed to the high turnover rate of basal structures, such as stipes, which may compensate better for the detrimental effects of combined environmental factors (Gómez et al. 2005). The stipes analysed herein exhibited generally higher chlorophyll relative content than blades, pointing to a high metabolic activity of this algal tissue. On the other hand, a dominance of carotenoids was found in blades. Carotenoids play an important role in the function and structural integrity of the chloroplast thylakoid membrane, and blades are structurally defined by a much greater density of thylakoid and mitochondrial cristae *per unit* volume than stipes (Havaux 1998; Su et al. 2010). Thallus age, metabolic activity and physiological function, but also location of specific thallus parts in the water column, support the dynamic in pigment profiles (Gómez and Wiencke 1998; Stengel et al. 2011).

**Table 2** Carotenoid and chlorophyll contents in *L. ochroleuca*, *S. latissima* and *S. polyschides* extracts (in mg kg<sup>-1</sup> dry algae)

Samples	Carotenoids			Chlorophylls					Total											
	Fucox (1)			Fucox <i>cis</i> isom 1 (2)		Fucox <i>cis</i> isom 2 (3)		Violax (4)		Zeax (5)		β-Carot (6)		Chl <i>a</i> (7)		Pheoph <i>a</i> (8)		Chl <i>c</i> derivatives		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)		(9)	(10)									
Lo_W_N_Oct12	57.87 (0.89) <sup>a</sup>	4.12 (0.16) <sup>ab,w</sup>	3.35 (0.05) <sup>a</sup>	1.76 (0.07) <sup>ag,i</sup>	13.70 (0.29) <sup>a</sup>	0.91 (0.00) <sup>a</sup>	nd <sup>a</sup>	12.13 (1.89) <sup>a,d,i,j,k,l,m,s,v,z</sup>	nd <sup>a</sup>	60.81 (1.29) <sup>a</sup>	154.65 (4.64)									
Lo_B_N_Dec12	55.99 (0.53) <sup>ab</sup>	3.41 (0.18) <sup>b</sup>	3.05 (0.21) <sup>abc</sup>	4.00 (0.04) <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	15.99 (0.63) <sup>ab,k,s,v,z</sup>	nd <sup>a</sup>	nd <sup>b</sup>	82.44 (1.59)									
Lo_S_N_Dec12	4.39 (0.04) <sup>c</sup>	0.42 (0.02) <sup>c</sup>	0.38 (0.06) <sup>d</sup>	nd <sup>c</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	2.71 (0.14) <sup>b,c</sup>	36.06 (1.45) <sup>b</sup>	57.87 (4.01) <sup>abc</sup>	101.83 (5.72)									
Lo_W_N_Dec12	51.80 (1.09) <sup>ad</sup>	2.96 (0.07) <sup>ab,d,e,f</sup>	3.25 (0.52) <sup>a</sup>	3.49 (0.01) <sup>d</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	22.14 (0.54) <sup>b,d,k,r,s</sup>	nd <sup>a</sup>	nd <sup>b</sup>	83.64 (2.23)									
Lo_B_SBM_Jan13	34.01 (0.21) <sup>f</sup>	2.07 (0.02) <sup>d,g,h,i,j</sup>	2.36 (0.06) <sup>b,d,g,h,i</sup>	2.07 (0.03) <sup>g,i,l,p</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	6.46 (0.55) <sup>g,i,z</sup>	62.12 (0.09) <sup>c</sup>	102.99 (0.86) <sup>d</sup>	212.08 (1.82)									
Lo_S_SBM_Jan13	12.12 (1.76) <sup>ce</sup>	0.95 (0.04) <sup>eg</sup>	0.71 (0.15) <sup>gd</sup>	0.89 (0.09) <sup>e</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	3.67 (0.52) <sup>g,i,t</sup>	43.34 (0.04) <sup>d</sup>	35.70 (0.12) <sup>e</sup>	97.38 (2.72)									
Lo_W_SBM_Jan13	49.36 (0.31) <sup>bd</sup>	3.63 (0.09) <sup>ab,w</sup>	3.08 (0.24) <sup>abg</sup>	1.03 (0.14) <sup>eh</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	15.62 (0.40) <sup>a,d,h</sup>	73.89 (1.16) <sup>e</sup>	96.29 (0.91) <sup>d</sup>	242.90 (3.25)									
Lo_B_SBM_Jun13	163.65 (0.98) <sup>g</sup>	7.97 (0.00) <sup>ka</sup>	7.41 (0.11) <sup>ky</sup>	2.16 (0.08) <sup>ip</sup>	61.26 (0.81) <sup>c</sup>	4.56 (0.30) <sup>c</sup>	nd <sup>a</sup>	nd <sup>c,t,g,i</sup>	84.11 (3.63) <sup>f</sup>	109.46 (3.63) <sup>d</sup>	440.58 (9.54)									
Lo_B_Am_Apr13	161.71 (4.61) <sup>gh</sup>	9.56 (0.02) <sup>j</sup>	8.03 (0.15) <sup>k</sup>	1.72 (0.08) <sup>pa,j</sup>	15.73 (0.06) <sup>d</sup>	1.25 (0.04) <sup>d</sup>	nd <sup>a</sup>	nd <sup>c,t,g,i</sup>	62.58 (1.03) <sup>e</sup>	111.78 (2.17) <sup>d</sup>	371.11 (8.12)									
Lo_W_IMTA_Jan13	87.20 (0.54) <sup>ik</sup>	6.92 (0.11) <sup>km</sup>	6.69 (0.42) <sup>lm,y</sup>	2.80 (0.02) <sup>k</sup>	nd <sup>b</sup>	1.25 (0.04) <sup>d</sup>	18.23 (0.65) <sup>b</sup>	21.34 (4.65) <sup>eh,k,m,r</sup>	84.94 (5.18) <sup>f</sup>	102.94 (3.52) <sup>d</sup>	332.31 (15.13)									
Lo_B_IMTA_Apr13	153.89 (0.28) <sup>l</sup>	9.60 (0.17) <sup>j</sup>	9.70 (0.13) <sup>n</sup>	5.69 (0.31) <sup>l</sup>	nd <sup>b</sup>	0.90 (0.01) <sup>a</sup>	132.73 (6.00) <sup>c</sup>	nd <sup>c,t,g,i</sup>	144.44 (16.34) <sup>f</sup>	456.95 (23.24)										
SI_B(d5)_PV_Jul12	35.43 (0.16) <sup>l</sup>	2.16 (0.08) <sup>ej,no,p</sup>	1.93 (0.05) <sup>no,p,q</sup>	1.71 (0.02) <sup>am</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	6.90 (0.78) <sup>h,i,l,m</sup>	15.22 (0.15) <sup>g</sup>	11.66 (0.07) <sup>b</sup>	75.01 (1.31)									
SI_B(d10)_PV_Jul12	33.70 (0.11) <sup>f</sup>	3.00 (0.18) <sup>ab,o,q</sup>	2.38 (0.03) <sup>eh,j,p,r</sup>	1.81 (0.06) <sup>pa,n</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	nd <sup>c,t,g,i,n</sup>	nd <sup>a</sup>	nd <sup>b</sup>	40.89 (0.38)									
SI_B(d15)_PV_Jul12	20.32 (0.33) <sup>s,j</sup>	1.87 (0.07) <sup>fg,p,q,r</sup>	1.44 (0.12) <sup>ods</sup>	1.71 (0.03) <sup>oo</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	11.00 (1.24) <sup>a,d,i,j,k,l,m,o</sup>	nd <sup>a</sup>	nd <sup>b</sup>	36.34 (1.79)									
Lo_B_Am_Jan13	21.31 (0.18) <sup>l</sup>	1.50 (0.08) <sup>eh,h,r</sup>	1.96 (0.22) <sup>for,s</sup>	1.97 (0.01) <sup>pa,p</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	8.25 (0.41) <sup>h,d,i,j,k,l,m,p</sup>	nd <sup>a</sup>	nd <sup>b</sup>	34.99 (0.90)									
SI_W_IMTA_Jan13	89.56 (4.39) <sup>i</sup>	5.94 (0.02) <sup>ms</sup>	5.32 (0.08) <sup>tu</sup>	3.14 (0.39) <sup>dk</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	41.31 (3.89) <sup>q</sup>	nd <sup>a</sup>	nd <sup>b</sup>	145.27 (8.77)									
SI_B_IMTA_Apr13	126.01 (0.51) <sup>l</sup>	8.27 (0.26) <sup>ut</sup>	6.62 (0.47) <sup>uv</sup>	2.93 (0.18) <sup>k</sup>	nd <sup>b</sup>	1.44 (0.03) <sup>d</sup>	nd <sup>a</sup>	32.59 (0.71) <sup>qr</sup>	nd <sup>a</sup>	66.64 (1.61) <sup>a</sup>	244.50 (3.77)									
SI_W_IMTA_Apr13	87.56 (2.01) <sup>j</sup>	5.96 (1.21) <sup>mv</sup>	6.05 (0.37) <sup>mu,v</sup>	2.32 (0.32) <sup>lp</sup>	nd <sup>b</sup>	0.60 (0.11) <sup>e</sup>	nd <sup>a</sup>	17.62 (1.96) <sup>eh,m,o,p,s,t,u</sup>	nd <sup>a</sup>	120.11 (5.98)										
Sp_W_N_Jan13	79.11 (0.59) <sup>k</sup>	4.80 (0.02) <sup>s,w</sup>	4.68 (0.01) <sup>v</sup>	1.44 (0.01) <sup>gh,i,m,n,o</sup>	1.66 (0.05) <sup>e</sup>	3.32 (0.08) <sup>f</sup>	nd <sup>a</sup>	25.93 (6.95) <sup>eh,r,v</sup>	nd <sup>a</sup>	49.03 (0.58) <sup>ac</sup>	169.97 (8.29)									
Sp_W_IMTA_Jan13	235.95 (9.95) <sup>mm</sup>	14.33 (1.23) <sup>x</sup>	11.36 (0.07) <sup>w</sup>	nd <sup>c</sup>	nd <sup>b</sup>	2.66 (0.02) <sup>g</sup>	nd <sup>a</sup>	179.81 (19.80) <sup>w</sup>	nd <sup>a</sup>	245.10 (22.49) <sup>g</sup>	689.21 (53.56)									
Sp_B_IMTA_Apr13	58.61 (0.17) <sup>st</sup>	4.08 (0.12) <sup>ab,w</sup>	3.38 (0.13) <sup>ya</sup>	0.65 (0.03) <sup>e</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	9.53 (1.96) <sup>h,d,i,j,k,l,m,s,x</sup>	nd <sup>a</sup>	40.13 (0.26) <sup>ce</sup>	116.38 (2.67)									
Sp_S_IMTA_Apr13	92.14 (2.82) <sup>t</sup>	6.43 (0.29) <sup>m</sup>	5.20 (0.45) <sup>v</sup>	4.03 (0.04) <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>a</sup>	27.30 (0.55) <sup>h,q,r,u</sup>	nd <sup>a</sup>	64.26 (0.96) <sup>a</sup>	199.36 (5.11)									
Sp_W_IMTA_Apr13	166.37 (2.41) <sup>g</sup>	9.36 (0.03) <sup>z</sup>	8.87 (0.05) <sup>x</sup>	2.82 (0.18) <sup>k</sup>	nd <sup>b</sup>	2.31 (0.02) <sup>h</sup>	nd <sup>a</sup>	19.79 (0.25) <sup>eh,m,o,p,s,x,z</sup>	nd <sup>a</sup>	nd <sup>b</sup>	209.52 (2.94)									

Results are expressed as the mean (standard deviation) of three determinations. Different superscript letters denote statistically significant differences in the same column ( $p < 0.05$ ). Identity of samples according to Table 1

ng not quantified, nd not detected, *Fucox cis isom* fucoxanthin *cis* isomer, *Violax* violaxanthin, *Zeax* zeaxanthin,  $\beta$ -*Carot*  $\beta$ -carotene, *Chloroph* chlorophyll, *Pheoph* pheophytin

## Variations of the pigment composition of *S. latissima* tissues cultivated at different depths

Because environmental parameters change with depth, it is possible to predict that physiological responses of macroalgae, at a given moment, should vary along the gradient, resulting in significant metabolic changes. Previous reports have shown that the perennial kelp *S. latissima* can acclimate to different light and temperature conditions, within its limits of tolerance, by relying on different mechanisms, such as regulation of pigment levels (Davison 1987; Machalek et al. 1996; Andersen et al. 2013; Heinrich et al. 2015). As described above (“Materials and methods”), samples of *S. latissima* collected in the longlines cultivated at sea, in July, were subjected to decreasing light intensities and temperatures with increasing depth.

The total pigment amounts of *S. latissima* blade samples (samples SI\_B(d5)\_PV\_Jul12, SI\_B(d10)\_PV\_Jul12 and SI\_B(d15)\_PV\_Jul12) decreased along depth (5–15 m; Table 2). Overall, the carotenoid contents were not statistically different ( $p > 0.05$ ), except for fucoxanthin (1) and its *cis* isomer 2 (3) in blades collected at 15-m depth (SI\_B(d15)\_PV\_Jul12). Nevertheless, the largest variability was observed within chlorophyll pigments: blades harvested at 5-m depth (SI\_B(d5)\_PV\_Jul12) showed higher chlorophyll amounts than blades from deeper locations. Studies on photosynthesis and pigment composition under different temperature and light conditions revealed that algal acclimation patterns depend on the position of the photosynthetic tissue in the water column (Colombo-Pallota et al. 2006, Koch et al. 2016). Therefore, it seems that the ability of macroalgae to cope with specific or multiple environmental pressures depends on a combination of local adaptations.

## Conclusions

The chemical profile of an organism, population or community provides an alternative source of information to describe environmental conditions or impacts. Kelps are major primary producers in coastal ecosystems and well-known natural reactors with a huge metabolic plasticity. The three kelp species herein analysed appear to be suitable sources of pigments. Besides the considerable variability observed, the relative contents of carotenoids were generally higher than those of chlorophylls. Moreover, fucoxanthin was the dominant carotenoid. The interest in macroalgae as a source of biologically active metabolites, including carotenoids and chlorophylls, is steadily increasing. However, issues related to the continuous supply of macroalgae, and the natural variability that can occur in the same species and even within different parts of the same thallus, are a challenge to be overcome in programmes of marine natural product drug discovery and development.

Our results point to the complexity of the influence of different external and species-specific factors on pigment composition, opening doors for the potential use of IMTA systems to optimize the production of high-value chemicals with pharmaceutical and nutraceutical applications.

**Acknowledgments** The authors thank Diogo Silva, Patrícia Oliveira and Tânia Pereira (CIIMAR/CIMAR) for assistance in the cultivation and collection of the algae material. This work received financial support from the National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia/Ministério da Educação e Ciência) through projects UID/QUI/50006/2013 and UID/Multi/04423/2013, co-financed by the European Union (FEDER under the Partnership Agreement PT2020) and from the SeaweedStar/E\*6027 (EUROSTARS) project. To all financing sources the authors are greatly indebted. Fátima Fernandes (SFRH/BPD/98732/2013), Mariana Barbosa (SFRH/BD/95861/2013) and Andreia P. Oliveira (SFRH/BPD/96819/2013) thank FCT/MEC for the grants. Isabel C. Azevedo (SEAWEEDTECH/BPD/2011/02) thanks SeaweedTech/ES466813 project (Norwegian Research Council) for the grant.

## References

- Abreu MH, Pereira R, Yarish C, Buschmann AH, Isabel Sousa-Pinto I (2011) IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture* 312:77–87
- Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar Biol* 140:1087–1095
- Andersen GS, Pedersen MF, Nielsen ST (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J Phycol* 49:689–700
- Azevedo IC, Marinho GS, Silva DM, Sousa-Pinto I (2016) Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at Southern European climate conditions: growth and nutrient uptake at high temperatures. *Aquaculture* 459:166–172
- Barrington K, Chopin T, Robinson S (2009) Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In: Soto D (ed) *Integrated mariculture: a global review*. FAO Fisheries and Aquaculture Technical Paper No. 529. FAO, Rome, pp 7–46
- Bianchi TS, Kautsky L, Argyrou M (1997) Dominant chlorophylls and carotenoids in macroalgae of the Baltic Sea (Baltic proper): their use as potential biomarkers. *Sarsia* 82:55–62
- Boderskov T, Schmedes PS, Bruhn A, Rasmussen MB, Nielsen MM, Pedersen MF (2015) The effect of light and nutrient availability on growth, nitrogen, and pigment contents of *Saccharina latissima* (Phaeophyceae) grown in outdoor tanks, under natural variation of sunlight and temperature, during autumn and early winter in Denmark. *J Appl Phycol* 28:1153–1165
- Colombo-Pallota MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blades from different depths. *J Phycol* 42:1225–1234
- Connan S, Delisle F, Deslandes E, Gall EA (2006) Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. *Bot Mar* 49:39–46
- Davison IR (1987) Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *J Phycol* 23:273–283
- Fiuza AFD, Demacedo ME, Guerreiro MR (1982) Climatological space and time-variation of the Portuguese coastal upwelling. *Oceanol Acta* 5:31–40

- Gammone MA, Riccioni G, D'Orazio N (2015) Carotenoids: potential allies of cardiovascular health? *Food Nutr Res* 59:26762
- Gerasimenko NI, Skriptsova AV, Busarova NG, Moiseenko OP (2011) Effects of the season and growth stage on the contents of lipids and photosynthetic pigments in brown alga *Undaria pinnatifida*. *Russ J Plant Physiol* 58:885–891
- Gómez I, Wiencke C (1998) Seasonal changes in C, N and major organic compounds and their significance to morphofunctional processes in the endemic Antarctic brown alga *Ascoseira mirabilis*. *Polar Biol* 19:115–124
- Gómez I, Ulloa N, Orostegui M (2005) Morpho-functional patterns of photosynthesis and UV sensitivity in the kelp *Lessonia nigrescens* (Laminariales, Phaeophyta). *Mar Biol* 148:231–240
- Goss R, Jakob T (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* 106:103–122
- Hamid N, Ma Q, Boulom S, Liu T, Zheng Z, Balbas J, Robertson J (2015) Seaweed minor constituents. In: Tiwari BK, Troy DJ (eds) *Seaweed sustainability: food and non-food applications*. Academic, San Diego, pp 193–242
- Havaux M (1998) Carotenoids as membrane stabilizers in chloroplasts. *Trends Plant Sci* 3:14–151
- Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2015) Temperature and light interactively modulate gene expression in *Saccharina latissima* (Phaeophyceae). *J Phycol* 51:93–108
- Henley WJ, Dunton KH (1995) A seasonal comparison of carbon, nitrogen, and pigment content in *Laminaria solidungula* and *L. saccharina* (Phaeophyta) in the Alaskan Arctic. *J Phycol* 31:325–331
- Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) *Seaweed ecology and physiology*, 2nd edn. Cambridge University Press, Cambridge
- Ibañez E, Cifuentes A (2013) Benefits of using algae as natural sources of functional ingredients. *J Sci Food Agric* 93:703–709
- Koch K, Thiel M, Hagen W, Graeve M, Gómez I, Jofre D, Hofmann LC, Tala F, Bischof K (2016) Short- and long-term acclimation patterns of the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae) along a depth gradient. *J Phycol* 52:260–273
- Kostas ET, White DA, Du C, Cook DJ (2016) Selection of yeast strains for bioethanol production from UK seaweeds. *J Appl Phycol* 28:1427–1441
- Kumari P, Kumar M, Reddy CRK, Jha B (2013) Algal lipids, fatty acids and sterols. In: Domínguez H (ed) *Functional ingredients from algae for foods and nutraceuticals*. Woodhead Publishing, Cambridge, pp 87–134
- Küppers U, Kremer BP (1978) Longitudinal profiles of carbon dioxide fixation capacities in marine macroalgae. *Plant Physiol* 62:49–53
- Lanfer-Marquez UM, Barros RMC, Sinnecker P (2005) Antioxidant activity of chlorophylls and their derivatives. *Food Res Int* 38:885–891
- Lemos RT, Pires HO (2004) The upwelling regime off the west Portuguese coast, 1941–2000. *Int J Climatol* 24:511–524
- Machalek KM, Davison IR, Falkowski PG (1996) Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant Cell Environ* 19:1005–1016
- Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. *J Appl Phycol* 27:1963–1973
- Martins CDL, Lhullier C, Ramlov F, Simonassi JC, Gouvea LP, Noernberg M, Maraschin M, Colepicolo P, Hall-Spencer JM, Horta PA (2014) Seaweed chemical diversity: an additional and efficient tool for coastal evaluation. *J Appl Phycol* 26:2037–2045
- Olischläger M, Iñiguez C, Gordillo FJ, Wiencke C (2014) Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased pCO<sub>2</sub> and temperature reveals ecotypic variation. *Planta* 240:1213–1224
- Oliveira AP, Lobo-da-Cunha A, Taveira M, Ferreira M, Valentão P, Andrade PB (2015) Digestive gland from *Aplysia depilans* Gmelin: leads for inflammation treatment. *Molecules* 20:15766–15780
- Peng J, Yuan JP, Wu CF, Wang JH (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar Drugs* 9:1806–1828
- Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *J Appl Phycol* 27:363–373
- Schmid M, Stengel DB (2015) Intra-thallus differentiation of fatty acid and pigment profiles in some temperate Fucales and Laminariales. *J Phycol* 51:25–36
- Schubert N, García-Mendoza E, Pacheco-Ruiz I (2006) Carotenoids composition of marine red algae. *J Phycol* 42:1208–1216
- Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecol Evol* 3:4016–4038
- Stahl W, Sies H (2003) Antioxidant activity of carotenoids. *Mol Aspects Med* 24:345–351
- Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environ Conserv* 29:436–459
- Stengel DB, McGrath H, Morrison LJ (2005) Tissue Cu, Fe and Mn concentrations in different-aged and different functional thallus regions of three brown algae from western Ireland. *Estuar Coast Shelf Sci* 65:687–696
- Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. *Biotechnol Adv* 29:483–501
- Su HN, Xie BB, Zhang XY, Zhou BC, Zhang YZ (2010) The supramolecular architecture, function, and regulation of thylakoid membranes in red algae: an overview. *Photosynth Res* 106:73–87
- Subramoniam A, Asha VV, Nair SA, Sasidharan SP, Sureshkumar PK, Rajendran KN, Karunakaran D, Ramalingam K (2012) Chlorophyll revisited: anti-inflammatory activities of chlorophyll *a* and inhibition of expression of TNF- $\alpha$  gene by the same. *Inflammation* 35:959–966
- Takaichi S (2011) Carotenoids in algae: distributions, biosyntheses and functions. *Mar Drugs* 9:1101–1118
- Wang Y, Xu D, Fan X, Zhang X, Ye N, Wang W, Mao Y, Mou S, Cao S (2013) Variation of photosynthetic performance, nutrient uptake, and elemental composition of different generations and different thallus parts of *Saccharina japonica*. *J Appl Phycol* 25:631–637
- Willstätter R, Page HJ (1914) Chlorophyll. XXIV. The pigments of the brown algae. *Justus Liebigs Ann Chem* 404:237–271
- Zorofchian Moghadamtousi S, Karimian H, Khanabdali R, Razavi M, Firoozinia M, Zandi K, Abdul KH (2014) Anticancer and antitumor potential of fucoidan and fucoxanthin, two main metabolites isolated from brown algae. *Sci World J* 2014:1–10