Seasonal and species-specific response of five brown macroalgae to high atmospheric CO_2

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ABSTRACT: To date, the impact of future increases in carbon availability on marine primary producers is uncertain due to the diversity of carbon acquisition strategies and differences in the ability of species to adapt to concurrent changes in other environmental factors. The aim of this study was to compare the CO_2 uptake rates of intertidal brown algae inhabiting different shore levels on the Irish west coast to increased atmospheric CO_2 levels with the use of an infra-red gas analyser. Considering the 5 species studied, 4 (*Pelvetia canaliculata*, *Ascophyllum nodosum*, *Sargassum muticum*, *Saccharina latissima*) showed a significant linear relationship between CO_2 uptake and CO_2 concentration with only *Fucus spiralis* exhibiting no increase. The lower shore species (*S. muticum*, *S. latissima*) exhibited statistically significant increases in V_{max} between CO_2 treatments and, in addition, a much larger affinity for carbon. The relative increase in carbon uptake and the slope of the CO_2 concentration versus CO_2 uptake curve (C_α) oscillated over the annual cycle for *A. nodosum* and *S. muticum*, and was also dependent on site in the case of *A. nodosum*, highlighting the importance of ambient environmental conditions on the relationship between availability and uptake. The affinity for CO_2 was correlated with *in situ* temperature in both species, suggesting a seasonal strategy of photosynthetic up-regulation. In the case of *A. nodosum*, the results indicated that future impacts of increased CO_2 would be greatest during the spring growth period. Future studies into the impact of high CO_2 levels on macroalgae need to consider the seasonal regulation of photosynthesis, as this will strongly influence the response observed.

KEY WORDS: Macroalgae · CO_2 · Photosynthesis · Intertidal · Climate change · Infra-red gas analyser

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INTRODUCTION

Without mitigation, anthropogenic emissions will lead to a continued rapid rise in atmospheric CO_2, until the end of the century (Meehl et al. 2007). Through absorption by the oceans, an increased availability of dissolved CO_2 for primary producers and an average decrease in global pH levels of marine waters is further projected (Roleda & Hurd 2012). Intertidal macroalgae, inhabiting the interface between the ocean, land and atmosphere, will be exposed to increased carbon availability during both emersion and submersion periods with potential implications for primary productivity and carbon uptake strategies (Giordano et al. 2005). The response of these primary producers is of specific interest (Raven 2011) due to their importance in elemental cycling, food webs and as a structural component of temperate coastal ecosystems (Steneck et al. 2002, Edgar et al. 2004).

The intertidal zone is characterised by dramatic environmental changes: on the one hand, emersed seaweeds benefit from higher light and carbon availability; while, on the other hand, extreme temperature changes, desiccation and the absence of a nutrient supply can negatively influence their photosynthesis and primary production (Maberly & Madsen 1990, Lobban & Harrison 1997). Despite the extreme conditions, some intertidal species, particularly Fucales, show higher photosynthetic rates during emersion than submersion (Johnston & Raven

1987, Surif & Raven 1990) and many are not considered to be saturated at present day CO_2 concentrations (Johnston & Raven 1987, Surif & Raven 1990, Gao et al. 1993, Zou & Gao 2002, 2004). Additionally, differences in dissolved inorganic carbon acquisition and levels of carbonic anhydrase have been shown between intertidal species (Giordano & Maberly 1989, Murru & Sandgren 2004).

The responses of intertidal macroalgae to increased atmospheric CO_2 are likely to have important consequences for their competitive ability and therefore the composition of future macroalgal communities (Hepburn et al. 2011). Their ability to act as a sink for CO_2 could also be altered, with implications for atmospheric concentrations, ocean–air exchanges and carbon cycling. The response direction and magnitude will depend on a number of environmental factors (temperature, irradiance and nutrients) which oscillate over both tidal and seasonal timescales. Macroalgae are known to acclimatise to ambient conditions through alterations of internal pigments levels (Stengel & Dring 1998), and the regulation of enzymes such as ribulose-1,5-bisphosphate carboxylase (RUBISCO) (Davison & Davison 1987). Furthermore, the adaptation of macroalgae to the emersion environment, which defines their position on the littoral zone, may play a role in determining their affinity for increasing CO_2 availability (Surif & Raven 1990).

Using algae from the Irish west coast, we experimentally investigated the hypothesis that the affinity of intertidal brown algae to increasing levels of CO_2 will vary between different macroalgae species found along the continuum of the intertidal zone, and the response of intertidal species to increased CO_2 levels will change over the annual cycle in response to changes in environmental factors such as temperature. Five species (*Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum nodosum*, *Sargassum muticum*, *Saccharina latissima*) present in abundance along a vertical gradient from the high to low intertidal areas, respectively, of the North Atlantic region were studied. These species are naturally exposed to large environmental changes over tidal and seasonal cycles; however, their comparative response to increased atmospheric CO_2 during exposure remains unclear. The algae were exposed to CO_2 levels up to and including 800 ppm, which is considered the high end of the IPCC model predictions of future atmospheric concentrations in 2100 (Meehl et al. 2007). Differences in their mechanistic response to atmospheric CO_2 levels could give insight into the future competitive ability of the individual species and community responses to increased CO_2.

For 2 canopy forming species (*Ascophyllum nodosum* and *Sargassum muticum*), potential seasonal variations in response to higher CO_2 availability were determined in greater detail. The indigenous *A. nodosum* and the invasive *S. muticum* both play important roles in community structure and functioning. They exhibit differences in terms of growth and ecological traits, and their comparative response to climate change factors over seasonal cycles has not been previously investigated. *A. nodosum* is an abundant and widely distributed species found on the mid-intertidal area of sheltered North Atlantic coasts (Cousens 1984). It is a structurally important species, and its growth correlates well with mean annual seawater temperature, meaning it is sensitive to environmental change (Keser et al. 2005). *S. muticum* has successfully invaded lower intertidal to subtidal regions of Irish coastal areas since its first record in 2001 (Loughnane & Stengel 2002). It exhibits much faster growth rates than *A. nodosum* and has, interestingly, been shown to thrive close to natural CO_2 vents, showing a resistance to high amounts of CO_2 (Hall-Spencer et al. 2008).

Finally, the response of *Ascophyllum nodosum* CO_2 uptake to combined temperature and CO_2 alterations was determined for 3 seasons at 2 study sites. This experiment explored the influence of these 2 factors against a background of changing *in situ* environmental parameters and, therefore, inherent seasonal adaptation strategies.

The objectives of this study were hence (1) to determine the eco-physiological impact of increased CO_2 availability on CO_2 uptake (CO_2 uptake–irradiance [CO_2 uptake–E] curves, CO_2 uptake–CO_2 curves) of 5 intertidal phaeophyceaen species (*Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum nodosum*, *Sargassum muticum*, *Saccharina latissima*); (2) to assess the seasonal variation in the relationship between CO_2 concentration and light-saturated CO_2 uptake through CO_2 uptake–CO_2 curves for 2 species (*A. nodosum*, *S. muticum*) with replication between 2 study sites on the Irish west coast; and (3) to determine the influence of temperature on the assimilation of ambient and increased CO_2 concentrations on *A. nodosum* over different seasons.

MATERIALS AND METHODS

Algal collection and storage

Algal tissue of the 5 species considered in this study (*Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum no-*

dosum, Sargassum muticum, Saccharina latissima) were collected from 2 comparable exposed rocky shore study sites located on the west coast of Ireland, Finavarra (53° 09′ 25″ N; 09° 06′ 58″ W), County Clare and Letterard (53° 36′ 64″ N, 09° 88′ 87″ W), County Galway. In most cases, meristematic tissue was sampled; however, for *S. muticum*, the growing frond (containing leaves, central stalk and vesicles) was taken. Before experimentation, all specimens were acclimatised in the laboratory at 15°C under low light (50 µmol photons m^{-2} s^{-1}, fluorescent lighting) on a 12 h:12 h light:dark cycle for 24 to 48 h in air-bubbled water collected at the respective study sites. Temperatures at the mid-tide level of both study sites were continuously measured using StowAway TidbiT temperature data loggers (Onset). Water temperature was recorded every 10 min over 12 mo by data loggers fixed with cable ties to rocks within the *A. nodosum* bed.

CO$_2$ uptake measurements

CO_2 exchange was measured using an infra-red gas analyser (IRGA; LCpro+, ADC Bioscientific), equipped with a measuring cone chamber containing an LED light source, which allowed the regulation of light level, temperature, CO_2 and humidity. For all experiments, the temperature within the chamber was kept constant at 15°C, CO_2 at 400 or 800 ppm and the maximal humidity achievable within the chamber (approximately 53%) was set to avoid desiccation of the samples.

Tissue of each algal species was exposed to each light or CO_2 step, depending on the experiment, for a period of 30 min. The change of CO_2 level between the inflow and outflow of the chamber was calculated every minute. Results were considered between 20 and 30 min after exposure to air; this is considered to be the time period when photosynthesis is at its maximum following exposure (Johnston & Raven 1987, and references therein). Each CO_2 uptake–irradiance curve was undertaken in triplicate for each species. Algae were subsequently freeze-dried for 48 h (Labconco FreeZone 6 L Freeze dry system) and photosynthetic rates were expressed per gram dry weight (DW).

Through exposure to a series of 7 light steps (0 to 1000 µmol photons m^{-2} s^{-1}), CO_2 uptake–*E* curves were produced for 3 replicates of each species (n = 3) at 400 and 800 ppm to determine photosynthetic parameters of carbon assimilation under emersed conditions. Carbon uptake (µmol C g^{-1} DW s^{-1}) was plotted as an exponential function of irradiance (µmol photons m^{-2} s^{-1}) for all data using the model of Webb et al. (1974).

$$CO_2 \ \text{uptake} = V_{\max}\left[1 - \exp\left(\frac{-E}{E_k}\right)\right] \qquad (1)$$

Where $V_{\max}$ is the maximum rate of carbon uptake (µmol C g^{-1} DW s^{-1}), and E_k is the irradiance at which CO_2 uptake is maximal (µmol photons m^{-2} s^{-1}).

Photosynthetic carbon exchange was determined over a range of 8 carbon concentrations from 400 to 800 ppm, again using 3 separate individuals (n = 3). CO_2 uptake–CO_2 curves were undertaken using the IRGA at a constant measuring temperature of 15°C, saturating photosynthetically active radiation (PAR) levels (500 µmol photons m^{-2} s^{-1}, Lüning 1981) and relative humidity (R.H., 53%) as above. As with the CO_2 uptake–*E* curves, meristematic tissue (or frond in the case of *Sargassum muticum*) was exposed to each light step for a 30-min period, with only values from the final 10 min of sampling being used. The relationship between CO_2 concentration and CO_2 uptake was determined using linear analysis methods and the slope of the curve (C_α) was considered a measure of the efficiency of the algae in utilising the increasingly available CO_2.

Impact of increased CO$_2$ on the photosynthesis of five intertidal macroalgae

Comparative studies on 5 species from different shore levels were undertaken on *Pelvetia canaliculata* (L.) Decaisne & Thuret, *Fucus spiralis* (L.), *Ascophyllum nodosum* (L.) Le Jolis, *Sargassum muticum* (Yendo) Fensholt and *Saccharina latissima* (L.) Lane, Mayes, Druehl & Saunders collected from Letterard between October and December 2011. CO_2 uptake–*E* curves were carried out at 2 different CO_2 levels (ambient and 800 ppm) to determine the influence of CO_2 availability on photosynthesis curves. CO_2 uptake–CO_2 curves were also undertaken to elucidate the influence of CO_2 on maximum photosynthetic rates.

Seasonal influence of increased CO$_2$ on photosynthesis of *Ascophyllum nodosum* and *Sargassum muticum*

Ascophyllum nodosum tips and *Sargassum muticum* fronds were collected monthly during spring and neap tide periods between February 2010 and January 2011 from both sites to determine the seasonal influence of increased CO_2 availability on their CO_2 uptake over the annual cycle. CO_2 uptake–CO_2 curves were undertaken, as above, between 400 and 800 ppm at saturating PAR levels.

Seasonal temperature influence on *Ascophyllum nodosum*

In April and October 2011, and January 2012, assimilation of CO_2 at 400 and 800 ppm by *Ascophyllum nodosum* from both study sites was recorded at 4 temperatures (5, 10, 15 and 20°C) to determine seasonal variations in responses to temperature at saturating PAR levels. The temperatures chosen were close to average seasonal conditions in winter (5°C) spring/autumn (10°C) and summer (15°C), while 20°C was at the upper level of summer average temperatures. Algal tips were collected as for other experiments.

Statistical analysis

Statistical analyses were undertaken using SPSS V.21. All data were tested using Shapiro-Wilk test for normality and Levene's test of equality of error variances before statistical analysis; when normality was not found, data were log transformed. The effect of light and CO_2 level on carbon uptake over the CO_2 uptake–E curve was undertaken using 2-way ANOVAs for each species. The effect of species and CO_2 level on CO_2 uptake parameters V_{max} and I_k (log transformed) were undertaken using a 2-way ANOVA. Following this, the effect of CO_2 level on the V_{max} for each individual species was completed using 1-way ANOVAs. Linear regression analysis and 1-way ANOVAs were used to look at the relationship between CO_2 concentration and CO_2 uptake for the 5 species studied. To test the influence of month, CO_2 level and site on CO_2 uptake at 400 and 800 ppm, mixed-effect ANOVAs were performed on each species with month and CO_2 level as fixed fac-

tors and site as a third random effect. Similarly, mixed-effect ANOVAs were used to determine the influence of month and site on the calculated C_α values. Following this, polynomial models were fitted to the *Ascophyllum nodosum* and *Sargassum muticum* C_α data to explore the seasonal oscillation in the affinity for CO_2. Linear models were used to assess the relationship between *in situ* temperature and C_α for the 2 species at the 2 study sites. Finally, 3-way ANOVAs were used to look at the influence of month, CO_2 level and temperature on V_{max} for the 2 sites studied in the temperature effect experiments and were followed by Tukey Honestly Significant Difference (HSD) post hoc tests to look at differences between seasons.

RESULTS

Impact of increased CO_2 on the photosynthesis of five intertidal macroalgae

The results showed that in most cases the intertidal species studied reacted similarly to increased CO_2 levels. Carbon uptake was significantly influenced by both CO_2 ($p < 0.001$) and PAR level ($p < 0.05$) during the CO_2 uptake–E curves for all species (Fig. 1A–E), an interactive effect was not, however, found, indicating that the influence of CO_2 level was not dependent on the light step. A 2-way ANOVA on the calculated CO_2 uptake-E curve parameters indicated a significant influence of CO_2 ($F = 16.10$, $p < 0.001$) and species ($F = 16.26$, $p < 0.001$) on V_{max} (Table 1), but no significant interaction between these 2 factors was found, suggesting that the response to higher CO_2 was not dependent on species.

Table 1. CO_2 uptake–E curve parameters calculated using the Webb model (Eq. 1) at 400 and 800 ppm (errors represent standard deviations, n = 3). Effects of CO_2 concentration on the parameters were calculated using 1-way ANOVAs (*$p < 0.05$, **$p < 0.01$). C_α: slope of the CO_2 uptake–CO_2 curve of the 5 species of macroalgae investigated. Letters represent statistically similar results (1-way ANOVA followed by Tukey Honestly Significant Difference post hoc tests)

| Algae species	CO_2 (ppm)	V_{max} (µmol C g^{-1} DW h^{-1})	E_k (µmol photons m^{-2} s^{-1})	C_α (µmol C g^{-1} DW h^{-1} ppm^{-1} CO_2)
Pelvetia canaliculata	400	7.69 ± 1.26	39.16 ± 12.38	0.018 ± 0.007[a]
	800	13.51 ± 3.80	29.8 ± 12.15	
Fucus spiralis	400	9.17 ± 2.96	69.32 ± 34.95	0.020 ± 0.002[a]
	800	14.91 ± 3.54	58.47 ± 45.83	
Ascophyllum nodosum	400	5.25 ± 1.27	108.57 ± 67.71	0.019 ± 0.002[a]
	800	7.95 ± 1.16	136.62 ± 115.17	
Sargassum muticum	400	13.77 ± 0.94 **	65.98 ± 23.09	0.066 ± 0.024[b]
	800	19.48 ± 1.87	30.24 ± 18.63	
Saccharina latissima	400	13.56 ± 5.86 **	129.9 ± 40.97 *	0.060 ± 0.014[b]
	800	24.91 ± 1.87	28.88 ± 1.803	
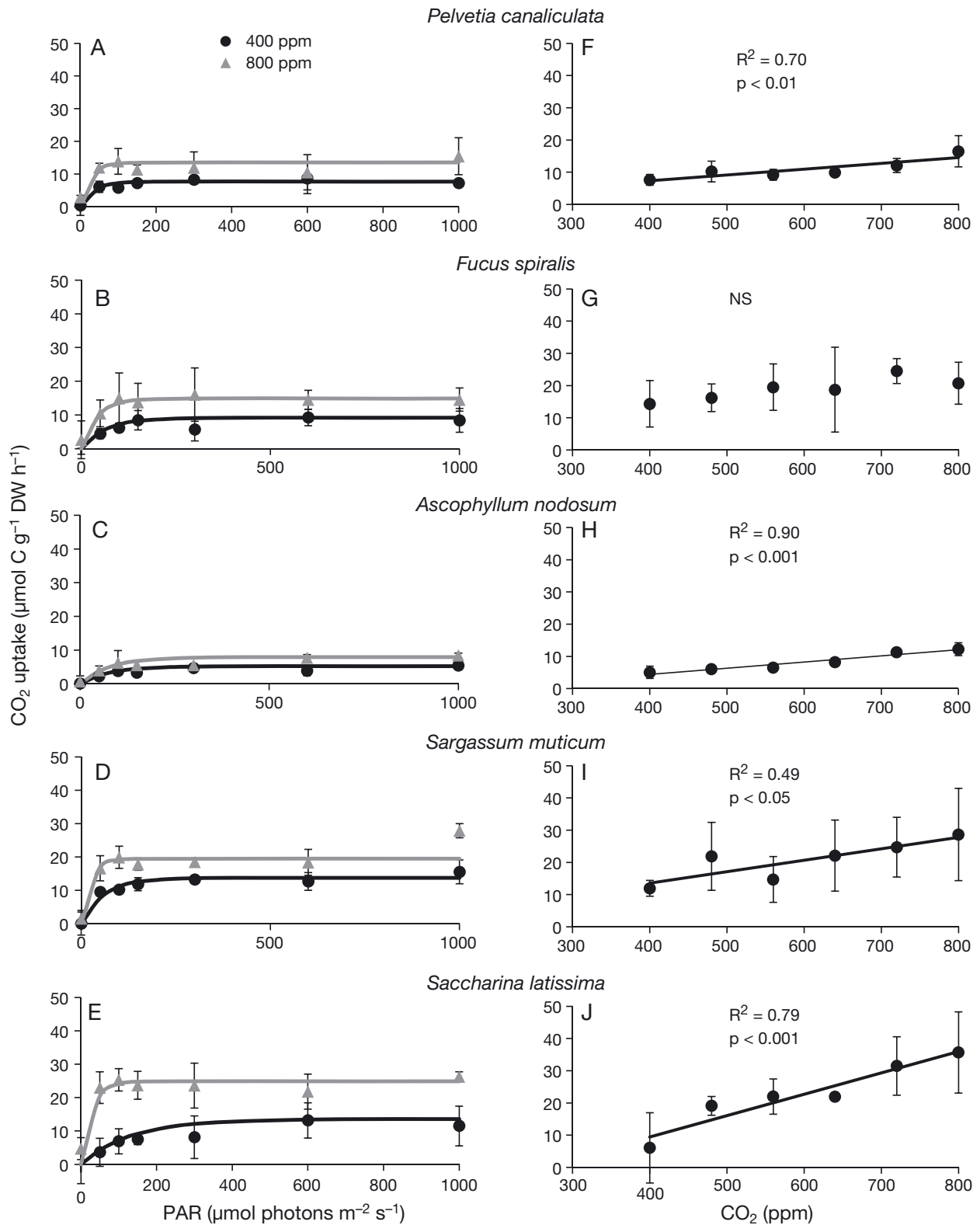
Fig. 1. (A–E) CO_2 uptake–E curves at 400 and 800 ppm for 5 species of macroalgae collected in October–November 2011. The average Webb model (Eq 1.) for each species at 400 and 800 ppm is represented by the black and grey lines respectively. (F–J) Rate of CO_2 uptake as a function of atmospheric CO_2 concentration. A linear model is represented by the black line. Error bars represent standard deviations (n = 3). NS: not significant

In the case of *Pelvetia canaliculata* and *Fucus spiralis*, CO_2 uptake became light saturated quickly during both CO_2 treatments, while no difference in E_k was evident (Fig. 1A,B, Table 1). *F. spiralis* exhibited similar rates of V_{max} at both ambient and increased CO_2 levels. During the sampling period studied (October–November), exposure to 800 ppm CO_2 had little influence on the maximal assimilation of carbon or the uptake efficiency of the mid-shore species *Ascophyllum nodosum*. Overall, this species exhibited the highest E_k values and lowest V_{max} during exposure to both ambient and increased CO_2 (Fig. 1C, Table 1).

The lower-shore species *Sargassum muticum* and *Saccharina latissima* had the highest values of V_{max} of all macroalgae studied during exposure to either ambient or high CO_2 conditions, indicating a faster assimilation of carbon during emersion than the upper- and mid-shore algae (Fig. 1D,E, Table 1). Results of 1-way ANOVAs for each species showed that V_{max} differed significantly between high and ambient CO_2 levels only in the case of *S. muticum* and *S. latissima* (Table 1). *S. latissima* exhibited an 83% increase in V_{max} between treatments, suggesting that this species is far from saturated under ambient conditions.

For E_k, the index of photoacclimation, 2-way ANOVAs showed no significant differences between species, but CO_2 did significantly influence this parameter ($F = 12.73$, $p < 0.001$). There was no interaction between species and CO_2 level. One-way ANOVAs conducted for each species revealed a significant influence of CO_2 only in the case of *Saccharina latissima* (Fig. 1E, Table 1), whereby light saturation of the CO_2 uptake–E curve occurred at lower E_k values than at ambient CO_2 levels. Saturation of the light curve occurred at the same light intensity in the remaining 4 species regardless of CO_2 level.

Carbon assimilation increased for 4 of the 5 species studied under elevated CO_2 levels (Fig. 1, Table 1); however, the affinity for carbon varied significantly between species. CO_2 uptake–CO_2 curves of all species except *Fucus spiralis* exhibited a significant linear relationship between CO_2 availability and uptake in the range of CO_2 concentrations tested, with a slope significantly different from zero (Fig. 1F–J). Excluding *F. spiralis*, none of the species appeared saturated up to 800 ppm. A 1-way ANOVA comparing the slopes (C_α) of the CO_2 uptake–CO_2 curves revealed differences between the lower-shore species and mid- and upper-shore species during treatments. The efficiency of *Sargassum muticum* and *Saccharina latissima* in assimilating carbon as CO_2 levels rose was significantly higher than for the other species examined (Table 1).
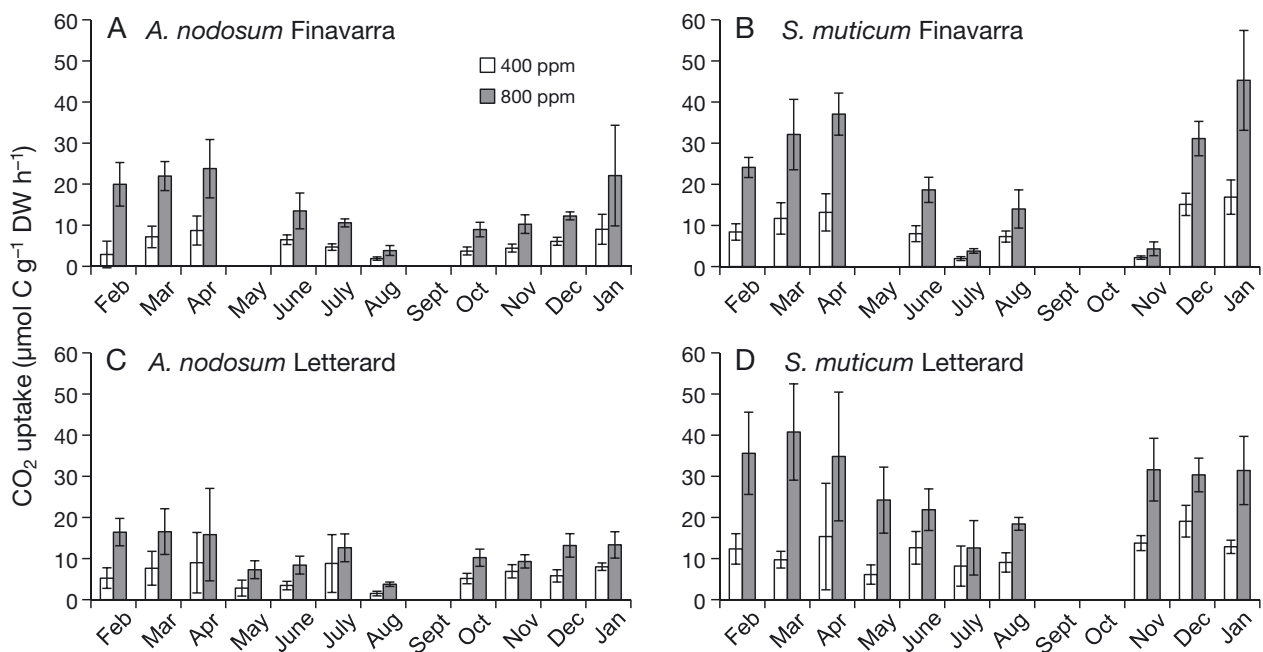

Fig. 2. Average monthly maximal carbon assimilation for (A,C) *Ascophyllum nodosum* and (B,D) *Sargassum muticum* at the 2 field sites on the Irish west coast (Finvarra and Letterard) when exposed to 400 and 800 ppm from February 2010 to January 2011. Experimental temperature was 15°C. Error bars represent standard deviations (n = 3)

Seasonal influence of increased CO_2 on
Ascophyllum nodosum and *Sargassum muticum*

Measured at constant light and temperature conditions, an increase in V_{max} values between 400 and 800 ppm was observed throughout the annual cycle for both *Ascophyllum nodosum* and *Sargassum muticum* at the 2 study sites (Fig. 2, data shown for 400 and 800 ppm only). In all cases, this relationship was linear, pointing towards a lack of saturation of the carbon uptake capacity during exposure. Univariate analysis showed a significant effect of CO_2 level (400 and 800 ppm) on both species (*A. nodosum*: $F = 189.32$, $p < 0.001$; *S. muticum*: $F = 90.51$, $p < 0.001$). *S. muticum* exhibited higher CO_2 assimilation rates than *A. nodosum* at both 400 and 800 ppm CO_2 levels during most months (excluding July, October and November).

A seasonal oscillation in V_{max} at both ambient and increased CO_2 levels was evident (Fig. 2) and indeed univariate statistical tests showed an effect of month on both species (*Ascophyllum nodosum*: $F = 18.50$, $p < 0.001$; *Sargassum muticum*: $F = 7.76$, $p < 0.001$). Generally, values were highest during late winter–early spring periods and decreased through spring–summer. Lowest values were measured during late summer periods and increased again in late autumn for both species (Fig. 2).

An influence of site on the V_{max} values recorded was only evident for *Ascophyllum nodosum* ($F = 13.65$, $p < 0.001$), but not for *Sargassum muticum*. Similarly, interactive effects of CO_2 level and month ($F = 5.76$, $p < 0.01$) and CO_2 level and site ($F = 14.74$, $p < 0.01$) were shown for *A. nodosum*, suggesting that both the time of the year and the differences in site had an impact on the response of *A. nodosum* to different CO_2 levels. In the case of *S. muticum*, no interactive effects of month, site and CO_2 level were shown, suggesting that the impact of increased CO_2 on V_{max} was not dependent on the time of year or site-specific conditions.

C_α followed a similar seasonal pattern and for both species, 2-way ANOVAs showed a significant influence of time (*Ascophyllum nodosum*: $F = 12.45$, $p < 0.001$; *Sargassum muticum*: $F = 4.03$, $p < 0.05$). In the case of *A. nodosum*, statistical differences between sites were also found ($F = 7.17$, $p < 0.05$). In both cases, no interaction was seen between the 2 parameters, suggesting that site did not have an impact on the monthly changes in C_α. A significant polynomial relationship between C_α and time was shown in all cases (Fig. 3). This relationship was, however, stronger for *A. nodosum* than *S. muticum*. C_α was significantly, negatively correlated with *in situ* temperature in the month preceding each measurement in all cases, with higher values found at lower *in situ*
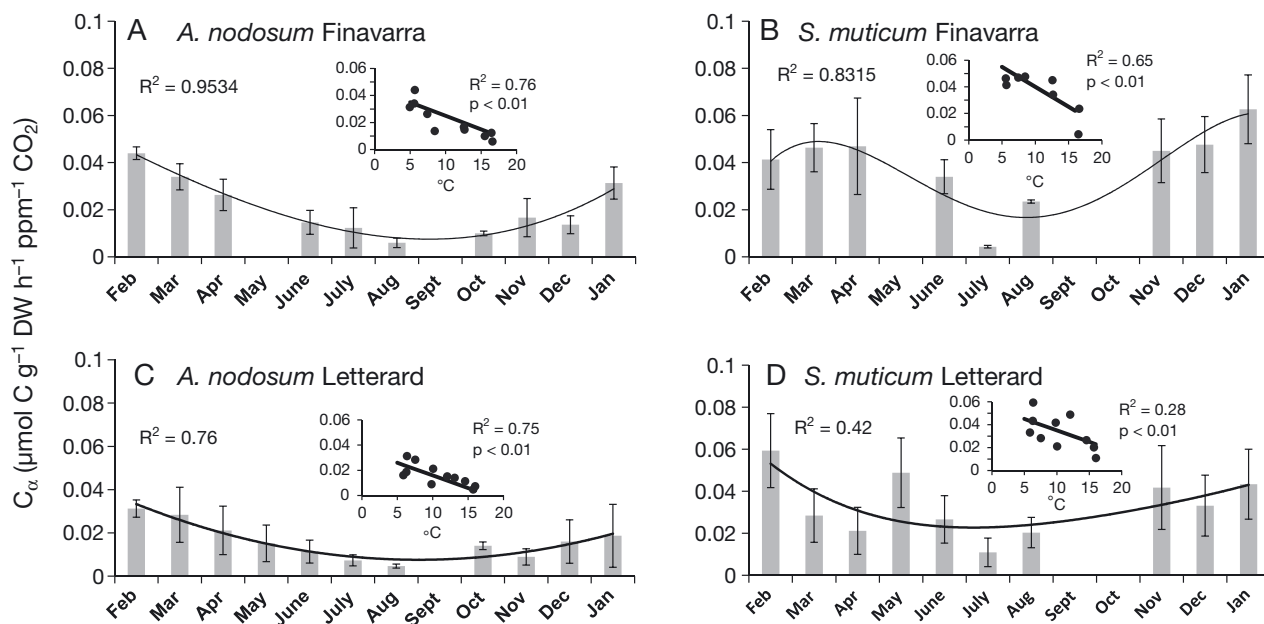

Fig. 3. Seasonal alterations in the slope of the CO_2 uptake–CO_2 curve (C_α) for (A,C) *Ascophyllum nodosum* and (B,D) *Sargassum muticum* from 2 study sites. Experimental temperature was 15°C. Error bars represent standard deviations (n = 3). Black line represents polynomial model fitted to the data. Inset into each graph shows the linear relationship between C_α and *in situ* temperature at the study site

growth temperatures (Fig. 3 insets). Again, this relationship appeared stronger for *A. nodosum* than for *S. muticum.* The calculated efficiency (C_α) of this invasive species in utilizing the increased CO_2 available was also consistently higher than that of *A. nodosum* (Fig. 3).

Seasonal temperature influence on *Ascophyllum nodosum*

The shape and relative magnitude of response to temperature at different CO_2 levels appeared similar at both CO_2 levels within each season for the 2 sites considered, but seasonal differences were evident (Fig. 4). Statistical analysis (3-way ANOVAs) showed that CO_2, temperature and season all influenced the V_{max} values individually (p < 0.001 in all cases). V_{max} increased with increasing temperature to 15°C in all seasons (Fig. 4). At 20°C, a decrease was, however, evident for *Ascophyllum nodosum* from both sites and CO_2 levels in winter, and at 400 and 800 ppm from Letterard samples in spring and at 800 ppm for Finavarra samples in spring. Tukey HSD post hoc tests showed that V_{max} values were statistically lower at all temperatures during autumn, followed by winter and
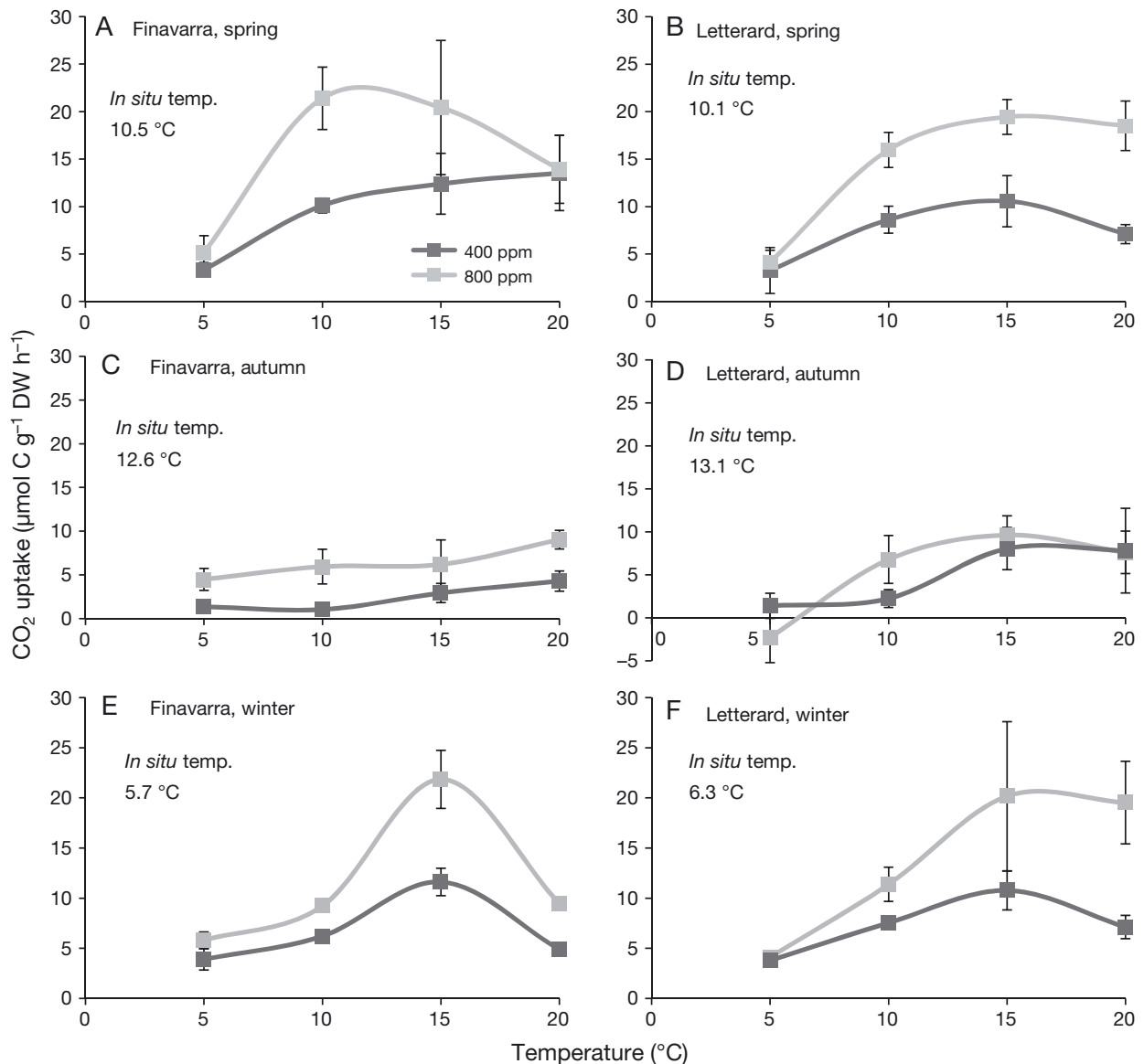

Fig. 4. Differences in CO_2 uptake rate during (A,B) spring, (C,D) autumn and (E,F) winter seasons at 400 and 800 ppm at 4 different temperatures for *Ascophyllum nodosum* from 2 sites. The average *in situ* temperature for each season and site is shown on each graph. Error bars represent standard deviations (n = 3)

then spring for Finavarra. V_{max} values for Letterard were lower in autumn, but statistically similar in spring and winter. The highest V_{max} values were observed in spring and winter periods, suggesting that, regardless of *in situ* temperature, highest carbon assimilation would occur during these periods at increased atmospheric concentrations. Interactive effects were found between CO_2 and temperature ($p < 0.005$ in both cases) for the 2 sites, and CO_2, temperature and season ($F = 7.27$, $p < 0.001$) for Finavarra.

DISCUSSION

Through exposure to different light, temperature and CO_2 regimes, we explored responses of 5 brown algae from different shore heights to predicted future high levels of CO_2. The results indicated that, at the sampling period studied (autumn), the affinity for carbon was species-specific and dependent on algal group (fucoid vs. non-fucoid). In the case of *Ascophyllum nodosum* and *Sargassum muticum*, season and for *A. nodosum,* site played a role in altering the affinity of the macroalgae for CO_2. While the impact of increasing CO_2 levels was not dependent on light availability, both *in situ* and experimental temperature regimes appeared to interact with CO_2 level to define the carbon uptake response. This highlights the importance of considering seasonal adaptation strategies of macroalgal species on the impact of future CO_2 conditions.

Analysis of the CO_2 uptake–irradiance curve parameters showed no increase in either V_{max} or E_k values for the high- to mid-intertidal species, while statistically significant increases in V_{max} were observed for the low-shore *Saccharina latissima* and *Sargassum muticum*. While the CO_2 curves suggest that present-day CO_2 concentrations limited the photosynthetic rate of 4 of the 5 macroalgae studied, statistical differences in the slope of the CO_2 uptake–CO_2 curve (C_α) between the fucoid species and the other 2 species studied indicated that their affinity for atmospheric CO_2 was higher, certainly during the autumn period. The results do, therefore, suggest a notable species-specific response to increased CO_2 availability. The different responses may be related to the extent of bicarbonate (HCO_3^-) use during submersion (Axelsson & Usitalo 1988, Mercado et al. 1998, Snoeijs et al. 2002, Murru & Sandgren 2004). Certain species are considered to be more efficient at assimilating HCO_3^- than others due to CO_2-concentrating mechanisms (CCMs) involving active transport mechanisms and/or the presence of the enzyme car-

bonic anhydrase (CA) either internally or externally (Mercado & Niell 2000, Zou et al. 2004). Indeed, Giordano & Maberly (1989) found, on average, higher levels of external CA in *Pelvetia canaliculata* and *Fucus spiralis*, than *Ascophyllum nodosum* and lower levels still in *S. latissima*. However, while differences in efficiencies of CCMs in diverse species have been demonstrated in previous studies, caution is necessary when extrapolating these to the entire annual cycle as the results from the seasonal study indicate that responses can vary considerably.

A seasonal oscillation in carbon uptake rates by *Ascophyllum nodosum* and *Sargassum muticum* at 15°C suggests an acclimation to *in situ* conditions, which allows these species to maintain photosynthetic rates throughout the annual cycle, as previously described by Stengel & Dring (1998) and Davison (1991). In winter and spring, when the algae were acclimatised to average temperatures of 5 to 10°C, the observed higher rates when measured at 15°C could, therefore, be a reflection of an up-regulation of carbon assimilation as a response to low *in situ* temperatures during this period. The experiments further indicate that exposure to increased CO_2 during emersion would result in a potentially higher uptake over the entire annual cycle. The results suggest that seasonal acclimation probably relating to *in situ* temperature oscillations will also determine the response magnitude of *A. nodosum* to CO_2, as shown by seasonal changes in C_α, and that this can differ between sites. While site-dependent responses certainly require more exploration, the possible influence of temperature clearly indicate that the effect of any CO_2 rise will need to be contextualised within the environment in which these macroalgal species reside.

In the case of *Sargassum muticum*, a more robust response was seen, as the increase in uptake with CO_2 level was similar regardless of time of year or site. Furthermore, the polynomial seasonality of C_α and the relationship between C_α and temperature although statistically significant was not as evident, and may relate to different types of algal tissue being included in the measurements as the morphology of the algae changes during the development period. Photosynthetic activity differs in various parts of the *Sargassum* frond with highest activity occurring in the leaves, followed by the receptacles, vesicles and holdfast (Gao & Umezaki 1989, Gao et al. 1991, Zou et al. 2011). In Ireland, development of the reproductive tissue of *S. muticum* occurs between April and September (Baer & Stengel 2010), so that materials used during the spring and summer period for

CO_2 exchange measurements would have included reproductive tissue (receptacle) and also vegetative (blades and air bladders) structures of the frond and would have been influenced by the relative composition of these structures. Furthermore, from early autumn through the winter, reproductive tissue is absent while seasonal growth rate is highest (Baer & Stengel 2010), which could have also contributed to the greater seasonal variability observed.

The observed seasonal differences in relative carbon uptake responses at both ambient and increased atmospheric CO_2 in *Ascophyllum nodosum* and seasonal C_α oscillations in both *A. nodosum* and *Sargassum muticum*, combined with the close relationship between *in situ* temperature and carbon uptake efficiency, highlight the important role of temperature and, hence, seasonal adaptation strategies in the response to CO_2. These oscillations probably relate to carboxylation of ribulose-1,5-bisphosphate by RUBISCO, which can be a limiting factor of primary production at light saturation (Farquhar & von Caemmerer 1982). Küppers & Weidner (1980) showed that for the brown alga *Laminaria hyperborea*, RUBISCO exhibits a circannual periodicity with a spring maximum similar to our higher C_α rates during this period. This enables this species to retain high primary productivity when *in situ* temperatures are still low and nutrient and light conditions are optimal. Regulation is thought to be related to phenotypical acclimation, with low temperatures leading to enhanced enzyme synthesis and/or high levels of nitrogen availability and associated uptake rates increasing the synthesis of proteins such as RUBISCO (Küppers & Weidner 1980, Gordillo et al. 2001, 2003).

While the seasonal study exposed the inter-annual oscillations in CO_2 uptake capacity and affinity, the temperature experiment (Fig. 4) brought this to a more practical level in that it revealed the interactive nature of temperature and CO_2 level on the uptake of CO_2 for the intertidal species *Ascophyllum nodosum*. Recent experiments on submerged macroalgal assemblages have also demonstrated that the response to combined CO_2 and temperature changes will differ from those relating to CO_2 alone (Olabarria et al. 2013) and clearly these 2 factors need to be considered in combination. The results of the current study also indicate that seasonal factors will influence the response to combined CO_2 and temperature changes, again highlighting the complex interaction between environmental factors. The seasonal interactions reported in the current study need to be considered not only when determining the possible impacts of future changes in both CO_2 and temperature conditions in the context of experimental design, but also modelling studies of future scenarios and responses.

Over the annual cycle, *Ascophyllum nodosum* and *Sargassum muticum* both exhibited a doubling of maximum CO_2 uptake rates during exposure to high CO_2 levels. While comparable studies on uptake rates during immersion are required, the potential implications of such an increase for intertidal ecosystems, if coupled with an increase in growth rate, would include a possible increase in the assimilation of nutrients, the capture and storage of carbon, particularly by the perennial *A. nodosum*, and a potential increase in organic matter available for higher trophic levels. In the case of *S. muticum*, an increase in CO_2 availability would probably support its proliferation in temperate coastal zones with possible implications for indigenous species. In considering the seasonal oscillation in responses to CO_2 level, the greatest influence of increased CO_2 on *A. nodosum* would be in late winter–early spring and, hence, during the optimal nutrient conditions as temperatures and light availability begin to rise. In the case of *S. muticum*, it would appear that the increase would probably be more consistent across the entire annual period.

Any long-term implications must be considered within the context of possible photosynthetic adaptation due to a regulation of enzyme activity by both temperature and changing atmospheric and indeed seawater CO_2 levels. Carbon limiting conditions have been shown to increase external CA activity (Andría et al. 2000), while a decrease in pigment concentration and RUBISCO under increased submersed CO_2 conditions has been noted (García-Sánchez et al. 1994, Drake et al. 1997, Andría et al. 2000). The impacts of prolonged exposure to high atmospheric CO_2 during low tide periods remain undetermined. A theoretical analysis of RUBISCO kinetics indicates that CO_2 stimulation of photosynthesis can increase with temperature, thereby leading to a combined effect of increases in these 2 environmental parameters (Long 1991). A mechanistic understanding of temperature acclimation and the seasonal changes in enzyme activity (CA and RUBISCO) of intertidal species, combined with long-term adaptation experiments would expose pre-adapted sensitivities to future climate conditions. This would contribute to a more accurate understanding of potential changes in macroalgal functioning and indeed carbon capture and storage in macroalgae-dominated coastal ecosystems as a result of elevated CO_2 over relative timescales (Beardall et al. 1998, Connell & Russell 2010, Roleda & Hurd 2012).

In conclusion, the enhancement of CO$_2$ uptake upon exposure to increased CO$_2$ varies with species, and is furthermore dependent on seasonally oscillating environmental conditions and may also depend on site-specific conditions. What is further highlighted by this study is that differences must be considered in the context of seasonal oscillations in CO$_2$ uptake efficiency, probably related to seasonal changes in RUBISCO activity, as observed for both *Ascophyllum nodosum* and *Sargassum muticum*. As shown here, the capability of macroalgal species to adapt to *in situ* environmental conditions is great and this plasticity will govern their capacity for adjusting to future climates.

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