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Influence of light intensity and nutrient source on $\delta^{13}C$ and $\delta^{15}N$ signatures in *Ulva pertusa*

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ABSTRACT: Stable isotopes are increasingly used to infer sources of nutrient enrichment and trophic linkages in coastal marine systems, although the utility of these tools often depends upon a predictable expression of $\delta^{15}N$ and $\delta^{13}C$ signatures by primary producers. Accordingly, we examined how tissue $\delta^{15}N$ and $\delta^{13}C$ values change in the common coastal marine alga *Ulva pertusa* Kjellman under contrasting light and nutrient treatments. In Expt 1, we manipulated nitrogen and phosphorus enrichment and light availability in a factorial design and found that: (1) $\delta^{13}C$ values in the tissue of *U. pertusa* depended upon interactions between light and nutrient availability, and there was no clear, overarching relationship between tissue $\delta^{13}C$ values and growth rate; and (2) these effects yielded a substantial (11.6‰) range of variation in $\delta^{13}C$ values. In Expt 2, we manipulated natural light (shaded versus unshaded) and nitrogen form (nitrate versus ammonium) in a factorial design and found that (3) $\delta^{15}N$ of *U. pertusa* tissue was closely tied to $\delta^{15}N$ of source nitrogen under all treatments and (4) $\delta^{15}N$ differences between high and low light treatments were largest when *U. pertusa* was supplied with ammonium (3.7‰), relative to nitrate (0.8‰). The variation in $\delta^{13}C$ values has implications for studies that use stable isotopes to infer trophic relationships in coastal marine environments, where gradients in nutrient concentration and light availability are common. The comparatively small range of $\delta^{15}N$ values expressed in *U. pertusa* supplied with nitrate confirms that this species represents a good proxy for $\delta^{15}N$ of biologically available nitrogen in nitrate-dominated coastal seawater.

KEY WORDS: Environmental gradients · Fractionation · Nutrient enrichment · Nutrient uptake · Stable isotopes

INTRODUCTION

Natural abundances of carbon and nitrogen isotopes are now routinely used to infer food web linkages and to trace sources of nitrogen enrichment across a wide range of ecological systems. These applications of stable isotopes are of great benefit to many field-based ecological studies, although the utility of the overall approach hinges upon the tendency of isotopes of nitrogen and carbon (^{14}N versus ^{15}N and ^{12}C versus ^{13}C) to assimilate into tissues of organisms (and pass through ecological systems) in a predictable manner. Provided this occurs (and there is sufficient variability in stable isotopes among different source pools), researchers can use isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) to infer feeding patterns of consumers and the distribution of terrestrial carbon and nitrogen sources (e.g. Ben-David & Schell 2001, Dunton 2001, Usui et al. 2006, Fry 2006, Catenazzi & Donnolly 2007).

Many marine studies that employ stable isotope approaches will necessarily rely upon a known (or at least a consistent) relationship between $\delta^{15}N$ and $\delta^{13}C$ ratios in the tissues of marine algae (i.e. the primary producers) and those of inorganic sources of carbon and nitrogen in coastal environments. For stable isotope ratios to be useful as tracers of pollutants or energy flow through marine food webs, signatures

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associated with different primary producers (e.g. species of marine plants and algae) should be relatively consistent and distinct from one another (e.g. Raven et al. 2002).

For marine plants and algae, one of the processes that determines tissue isotope ratios (i.e. signatures), including among-species variability and relationships with concentrations in the surrounding seawater environment, is known as 'fractionation'; effectively, this is the pattern of discrimination between ^{14}N versus ^{15}N and ^{12}C versus ^{13}C that occurs as nitrogen and carbon are assimilated into tissues.

Relatively few studies have examined variability in nitrogen fractionation for marine macroalgae (but see Cohen & Fong 2005, Cornelisen et al. 2007). Previous work on other photoautotrophs suggests that light availability (Wada & Hattori 1978, Heikoop et al. 1998, Needoba & Harrison 2004), growth rate (McKee et al. 2002), nutrient availability (Waser et al. 1998b, McKee et al. 2002), turbulence (Trudeau & Rasmussen 2003) and nitrogen source (Waser et al. 1998a) may all have some effect on fractionation of nitrogen during its incorporation in photoautotrophs. Similarly, other studies have shown that fractionation of carbon by algae may vary as a function of the physical environment as well as taxonomic difference (Wiencke & Fischer 1990, Raven et al. 2002). In those species of algae capable of assimilating HCO_3^-, changes in irradiance may also alter the proportion of total carbon that is derived from HCO_3^-, rather than isotopically lighter $CO_{2\,(aq)}$, leading to changes in tissue $\delta^{13}C$ values (Kubler & Raven 1995, Cornelisen et al. 2007).

Studies that use nitrogen and carbon stable isotope signatures in coastal environments would therefore benefit from an improved understanding of the patterns, magnitude and drivers of variability in the isotope ratios of macroalgae. We addressed this issue with a pair of complementary laboratory-based experiments that explored variability in carbon and nitrogen isotope ratios, respectively. Our experiments were partly motivated by an assessment of nutrient enrichment from a coastal sewage discharge (described by Dudley 2007) in which the isotopic signatures exhibited by the common macroalga *Ulva pertusa* Kjellman were correlated with light availability (i.e. along a depth gradient) independently of a correlation with nitrogen concentration (Dudley 2007). In contrast, other studies from New Zealand have shown that tissue $\delta^{15}N$ in *Ulva* spp. at non-polluted open coastal sites has a limited range (6.7 to 8.8‰; Rogers 1999, 2003, Barr 2007) that is seasonally stable (Barr 2007), despite seasonal environmental change (including change in light). In the current study, we first examined how $\delta^{13}C$ values of *U. pertusa* tissue are affected by conditions of nitrogen availability and light during growth. In our second experiment, we examined the effect of nitrogen source (either nitrate or ammonium) and light conditions on *U. pertusa* $\delta^{15}N$ values.

MATERIALS AND METHODS

General conditions. Large (200 to 300 mm in length) specimens of *Ulva pertusa* were collected from intertidal sandflats at Otumoetai in Tauranga Harbour, northeastern New Zealand (37° 39.68′ S, 176° 08.46′ E). After transportation to the Leigh Marine Laboratory (36° 16′ S, 174° 48′ E), *U. pertusa* specimens were acclimated in a partially-shaded outdoor holding tank with a constant flow of coarse-filtered (200 µm) coastal seawater. For the following 2 experiments, conducted over 2 consecutive summers in January 2005 and January 2006, we maintained individual *U. pertusa* thalli (around 3 g each) in a set of up to 16 individual outdoor seaweed growing chambers. Each growing chamber had a seawater volume of 4.5 l and was supplied with a constant 1.2 l min^{-1} flow of seawater via a tipper bucket to create turbulent conditions as described by Barr et al. (2008). Factorial experimental treatments (randomly assigned to the growing chambers) consisted of either ambient natural light or shaded natural light, and (except for natural seawater treatments) nitrogen supplied as either ammonium or nitrate. Shading was achieved with 3 layers of 50% neutral density screen to attenuate ambient natural light by 81%. The amount of light reaching the surface of the thalli was measured as photosynthetically active radiation (PAR) using a biospherical scalar irradiance probe (model QSL 2100). For nitrogen addition treatments, either ammonium chloride or sodium nitrate was supplied from individual concentrated stock solutions at a constant rate to each growing chamber via a multichannel peristaltic pump. The nitrogen stock supply rate was calculated to give a final constant concentration of 10 µM in seawater, as described by Barr et al. (2008). To ensure that no secondary phosphorus limitation occurred when nitrogen was added, phosphate was also supplied (as potassium dihydrogen phosphate) to give seawater N:P of 10:1 (note that macroalgal growth becomes phosphorus limited with N:P values above 30: 1; Atkinson & Smith 1983). For the 'no nutrient addition' treatment, deionised water only was added at the same rate as nutrient stocks were added to nitrogen-addition treatments.

Growth rates for individual *Ulva pertusa* specimens were estimated by collecting each individual thallus from its growing chamber (at either 2 or 4 d intervals throughout the experiments), removing the excess surface water using a salad spinner and then recording its fresh weight. For each experiment, initial weight of

individual thalli was around 3 g. After weighing individual thalli, surplus tissue was trimmed to give a return weight to the growing chamber of around 3 g. Algal growth rates were estimated using an exponential growth equation as follows:

$$W_t = W_0 \times e^{\mu t} \quad (1)$$

where W_t is the weight of algae at time t, W_0 is the initial weight and μ is the specific growth rate (d^{-1}).

Expt 1: Effects of nutrient enrichment and light regime on $\delta^{13}C$ signatures. In a factorial experiment, we evaluated the effect of light availability (ambient natural light versus shaded natural light) and 10 μM ammonium addition (relative to available nitrogen in low nutrient natural seawater) on tissue N content and tissue $\delta^{13}C$ signatures in *Ulva pertusa*. After acclimation under 50% ambient light for a period of 14 d, *U. pertusa* specimens were maintained under experimental conditions for a further 14 d using 3 individual replicate thalli for each of the 4 treatments. For the duration of this experiment, from 11 to 25 January 2005 (mean ± SE) daily solar radiation and PAR were 27.5 ± 1.2 MJ m^{-2} and 1671.4 ± 64.9 μE m^{-2} d^{-1}, respectively. Seawater temperature was 18.7 ± 0.3°C, seawater dissolved inorganic nitrogen (DIN) concentration was 1.8 ± 0.1 μM (comprised of ammonium 0.7 ± 0.1 μM, nitrate 1.0 ± 0.1 μM and nitrite 0.1 ± 0.01 μM), and seawater phosphate concentration was 0.3 ± 0.4 μM.

Expt 2: Effects of nitrogen source and light regime on $\delta^{15}N$ signatures. In a second factorial experiment, the effect of light availability (ambient natural light versus shaded natural light) and nitrogen source (ammonium versus nitrate) on tissue N content and $\delta^{15}N$ signatures in *Ulva pertusa* tissue was evaluated. However, unlike the first experiment, the seawater that was used to supply experimental treatments was first stripped of ambient nitrogen with an algal nutrient scrubber. This was done to eliminate, or at least reduce, any effect of ambient (natural) nitrogen on *U. pertusa* tissue $\delta^{15}N$ signatures. Nitrogen stripping was accomplished by running coarse-filtered seawater through a longitudinal race (9 m long by 200 mm wide and 100 mm deep) containing *U. pertusa* thalli placed throughout its length (note that this *U. pertusa* functioned purely as a means of removing nitrogen from seawater and should not be confused with the experimental *U. pertusa*). Effectiveness of nitrogen removal was confirmed by measuring nutrient concentrations throughout experimental runs. Artificial nitrogen (ammonium chloride or sodium nitrate) of known $\delta^{15}N$ signatures (–5.50‰ and 3.95‰, respectively) was then added back to the treatment seawater as described above.

Eight acclimated *Ulva pertusa* specimens weighing approximately 3 g were maintained under the same ambient (unshaded) light conditions in individual growing chambers for a preincubation period of 20 d. During this time, they were supplied with artificial nitrogen of known $\delta^{15}N$ signatures, prior to initiating the experiment. However, 4 specimens were supplied with 10 μM ammonium and 4 were supplied with 10 μM nitrate. At 2 d intervals during the first 14 d of this period, *Ulva pertusa* specimens were trimmed back to 3 g and returned to their respective chambers. The amount trimmed was approximately equivalent to half (3 g) of the total tissue present after each 2 d growth period, and over the 20 d preincubation phase, we estimated a cumulative tissue turnover of >95% for each specimen. For the duration of the preincubation phase, from 10 to 29 January 2006, average daily solar radiation and PAR were 20.9 ± 2.0 MJ m^{-2}, and 1245 ± 116 μE m^{-2} d^{-1}, respectively. Average seawater temperature over this period was 19.7 ± 0.1°C.

At the start of the manipulative part of the experiment, the 8 individual *Ulva pertusa* specimens (4 supplied with ammonium and 4 supplied with nitrate) were each halved and trimmed to give 2 thallus sections of approximately 3 g each (note that thalli were not trimmed during the final 6 d to allow sufficient tissue for this). These pairs were then randomly allocated to either shaded or unshaded treatments, although the same nitrogen addition regimes (ammonium or nitrate) were maintained. There were 4 replicate thalli for each treatment (in a total of 16 separate growth chambers). At the end of this experiment, we quantified tissue N content and $\delta^{15}N$ ratios for experimental *U. pertusa*, using the methods described below. Growth rates of *U. pertusa* thalli during the experimental phase were also estimated as described above. For the duration of this experiment, from 30 January to 14 February 2006, average daily solar radiation and PAR were 21.4 ± 1.4 MJ m^{-2} and 1277 ± 81 μE m^{-2} d^{-1}, respectively. Average seawater temperature over this period was 20.6 ± 0.1°C. Ambient seawater ammonium and nitrate concentrations in natural ('unstripped') seawater averaged over the duration of the experiment were 0.5 ± 0.1 μM and 0.7 ± 0.1 μM, respectively. However, after seawater passed through the scrubber, average ammonium and nitrate concentrations were 0.3 ± 0.1 μM and 0.2 ± 0.1 μM, respectively.

Tissue nitrogen and stable isotope signatures. Samples of *Ulva pertusa* from each experimental replicate were stored frozen at –20°C, subsequently defrosted, cleaned of any epiphytes and epifauna, dried at 70°C in a drying oven and ground to a fine powder. All isotope samples were analysed using a Europa Geo 20/20 isotope ratio mass-spectrometer interfaced to an ANCA-SL elemental analyser. Duplicate samples of 1.8 mg of powder were loaded into tin capsules for analysis of organic carbon and nitrogen content and carbon and nitrogen isotopic composition. The standard analytical error between duplicate analyses was

lower than ±0.3‰ for $\delta^{15}N$ and ±0.1‰ for $\delta^{13}C$. Relative isotopic concentrations are reported as $\delta^{15}N$ values relative to an air standard, where:

$$\delta^{15}N‰ = \left(\frac{^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{air}}{^{15}N/^{14}N_{air}} \right) \times 1000 \quad (2)$$

All $\delta^{13}C$ values were normalised to PeeDee Belemnite using Europa flour, International Atomic Energy Agency (IAEA) Beet sugar and Australian National University (ANU) sucrose, where:

$$\delta^{13}C‰ = \left(\frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{PDB}}{^{13}C/^{12}C_{PDB}} \right) \times 1000 \quad (3)$$

Statistical analysis. We used 2-way analysis of variance (ANOVA) to test for differences in tissue-$\delta^{15}N$, $\delta^{13}C$ and nitrogen content resulting from experimental manipulations of nutrients and/or light. To test the effect of treatments on growth rates in *Ulva pertusa* thalli, we used 2-way ANOVA of mean growth rates of each specimen (i.e. averaged over the duration of the experiment). Tukey's post hoc tests were used for pairwise comparisons. Data were tested and conformed to assumptions of normality and homogeneity of variance. All values are given as mean ± SE.

RESULTS

Expt 1: Effects of nutrient enrichment and light regime on $\delta^{13}C$ signatures, tissue N and growth

Ulva pertusa tissue introduced to growth chambers on Day 0 of the experiment had tissue $\delta^{13}C$ signatures of –15.3 ± 0.4‰, and tissue-N of 1.1 ± 0.1%. Final tissue $\delta^{13}C$ signatures in *Ulva* were influenced by the combined effects of light and nutrient addition (interaction term: $F_{1,8} = 319$, $p < 0.001$) (Fig. 1A). Due to this interaction, there was no significant main effect of light treatment on $\delta^{13}C$ signatures ($F_{1,8} = 0.44$, $p = 0.53$), although specimens supplemented with ammonium had higher $\delta^{13}C$ signatures overall relative to those grown in ambient seawater ($F_{1,8} = 1108$, $p < 0.001$) (Fig. 1A). Final tissue N content of *U. pertusa* was elevated for specimens supplemented with ammonium ($F_{1,8} = 481$, $p < 0.001$) and shaded ($F_{1,8} = 38.4$, $p < 0.001$), and 2-way ANOVA suggested no interaction between nutrient supplements and light availability ($F_{1,8} = 0.34$, $p = 0.57$) (Fig. 1B).

In terms of growth, *Ulva pertusa* supplemented with ammonium grew faster than unsupplemented *U. pertusa* ($F_{1,8} = 52.3$, $p < 0.001$) (Fig. 1C). Shading resulted in significantly lower growth ($F_{1,8} = 31.4$, $p < 0.001$) (Fig. 1C), and a significant interaction term ($F_{1,8} = 18.2$, $p = 0.003$) indicates that the magnitude of growth effects attributable to nutrient enrichment was much greater in full sun relative to shaded treatments (Fig. 1C).

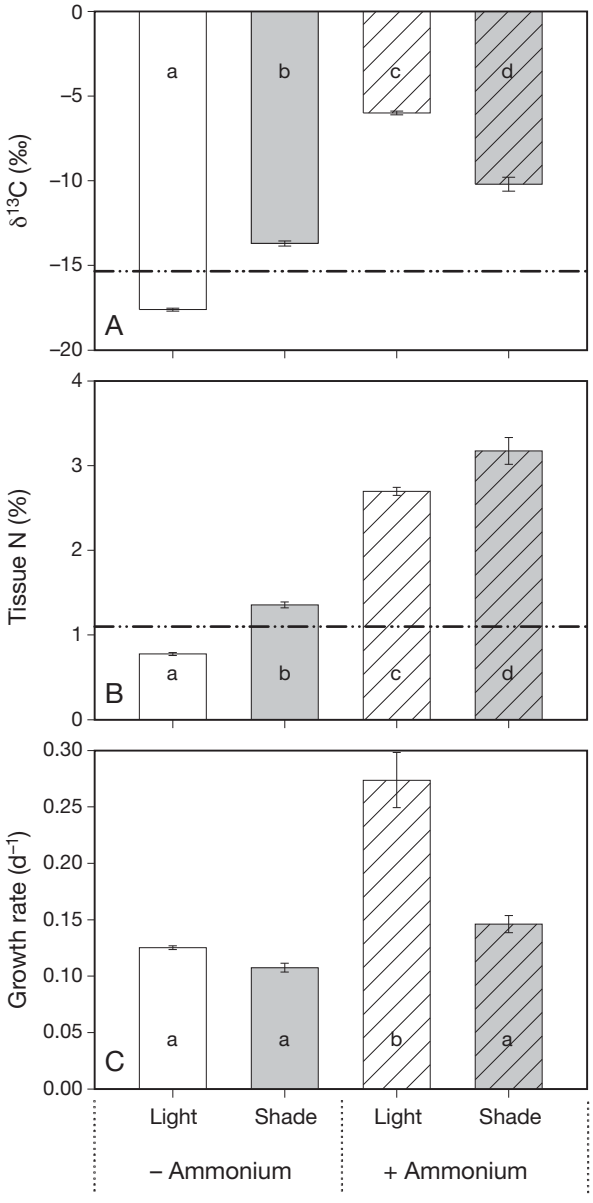

Fig. 1. *Ulva pertusa*. Effects of light and nitrogen addition on (A) final tissue $\delta^{13}C$ ratio, (B) tissue N content and (C) growth rate (Expt 1). Dot-dashed reference lines in (A) and (B) indicate tissue $\delta^{13}C$ and tissue N at the beginning of the experiment. Error bars show SE (n = 3). Bars labelled with the same lower case letter do not differ significantly ($p > 0.05$) according to Tukey's HSD method for pairwise multiple comparisons

Expt 2: Effects of nitrogen source and light regime on $\delta^{15}N$ signatures, tissue-N and growth

Ulva pertusa tissue introduced to growth chambers on Day 0 of the experiment (i.e. at the start of the preincubation phase) had tissue $\delta^{15}N$ of 7.8 ± 0.1‰, and tissue N of 0.9 ± 0.1%. At the start of the manipulative experiment, *U. pertusa* tissue had acclimatised to arti-

ficial (ammonium chloride or sodium nitrate) $\delta^{15}N$ signatures (–5.50 and 3.95‰, respectively). At this point, the nitrate-supplied *U. pertusa* had tissue $\delta^{15}N$ of 3.2 ± 0.1‰ and tissue-N of 2.7 ± 0.1%. Ammonium-supplied *U. pertusa* had tissue $\delta^{15}N$ of –6.3 ± 0.9‰ and tissue N of 3.0 ± 0.3% (Fig. 2A,B). During the experiment, fractionation of nitrogen isotopes during assimilation into *U. pertusa* tissue was influenced by the combined effects of light regime and nutrient form (interaction term: $F_{1,12} = 46.6$, $p < 0.001$) (Fig. 2A). Fractionation differed between ambient natural light and shaded light treatments ($F_{1,12} = 117$, $p < 0.001$), and there was no main effect of ammonium versus nitrate ($F_{1,12} = 2.23$, $p = 0.16$), although the significant interaction term (above) indicates that the effect of light is different for ammonium and nitrate treatments. The effect of light reduction on fractionation was greater for ammonium-supplied thalli than for nitrate-supplied thalli (Fig. 2A). Final tissue N content did not vary among *U. pertusa* treatments that were supplied with ammonium versus nitrate ($F_{1,12} = 0.03$, $p = 0.86$) (Fig. 2B). Tissue N content was greater in *U. pertusa* grown under shaded conditions relative to natural ambient light ($F_{1,12} = 180$, $p < 0.001$), and there was no significant interaction between light and nutrient treatments ($F_{1,12} = 0.37$, $p = 0.56$).

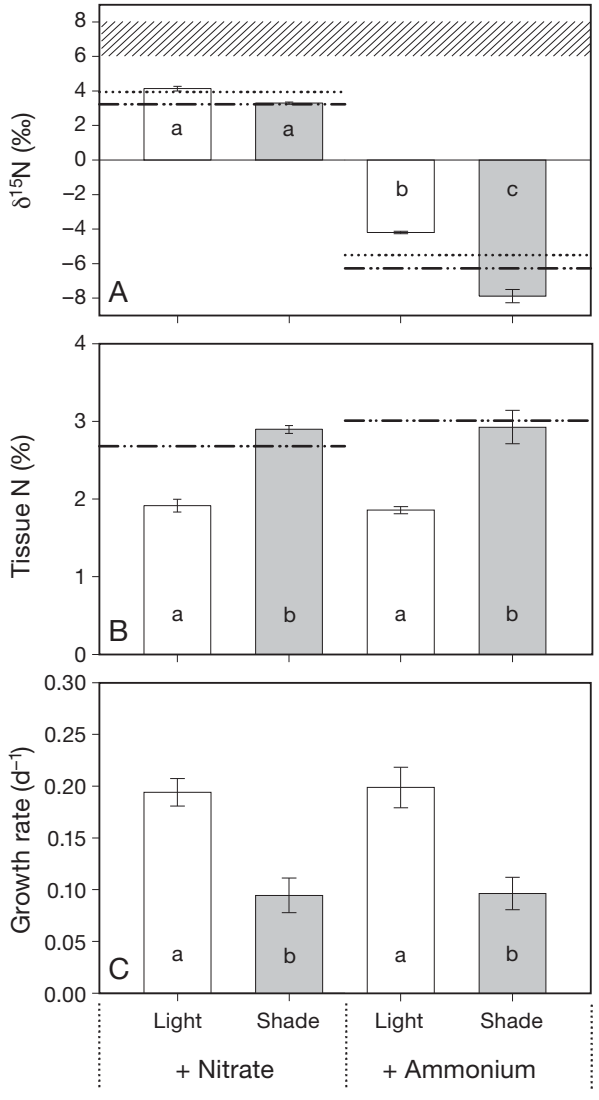

Fig. 2. *Ulva pertusa.* Effects of light, and nitrogen source and concentration on (A) tissue $\delta^{15}N$ ratio, (B) tissue N content and (C) growth rate (Expt 2). Dot-dashed reference lines in (A) and (B) indicate tissue $\delta^{15}N$ and tissue N at the beginning of the experiment. Dotted reference lines in (A) give $\delta^{15}N$ ratios for synthetic nitrate and ammonium sources. Cross-hatched panel in (A) shows $\delta^{15}N$ ratios for seawater dissolved inorganic nitrogen (see 'Discussion'). Error bars show SE (n = 4). Bars labelled with the same lower case letter do not differ significantly ($p > 0.05$) according to Tukey's HSD method for pairwise multiple comparisons

Similarly to the growth rates seen in Expt 1, *Ulva pertusa* that was grown under natural ambient light grew faster than that grown in shaded conditions ($F_{1,12} = 99.3$, $p < 0.001$; interaction term: $F_{1,12} = 0.02$, $p = 0.89$) (Fig. 2C). However, there was no difference in growth rate between nitrate- and ammonium-supplied *U. pertusa* ($F_{1,12} = 0.13$, $p = 0.73$) (Fig. 2C). Finally, it was also noted that the highest growth rates in *U. pertusa* were recorded in Expt 1 (compared to in Expt 2), and these may have been attributable to higher average levels of light measured in the first experiment (1671.4 µE m^{-2} d^{-1}) compared to those seen in the second experiment (1245 µE m^{-2} d^{-1}).

DISCUSSION

Stable isotope ratios of nitrogen, carbon and several other elements are now routinely used as tracers of nutrient sources and pathways, and to infer trophic relationships in marine ecosystems. Despite the clear utility and growing application of this approach, there is a paucity of information on the mechanisms (physiological and/or environmental) that affect the incorporation of these signals (particularly $\delta^{15}N$ ratios) into macroalgae (however, see Cohen & Fong 2005, Cornelisen et al. 2007). This information is essential for workers who aim to implement field studies and infer patterns of nutrient transfer in coastal marine systems from stable isotope ratios; such studies typically rely upon predictable transfer of both $\delta^{15}N$ and $\delta^{13}C$ ratios.

Effects of nutrient enrichment and light regime on $\delta^{13}C$ signatures

For $\delta^{13}C$ ratios, our experiments show that signatures depend upon light availability, and that these effects

are further mediated by the concentration of nitrogen available for uptake. We document a significant range of variation (11.6‰) in $\delta^{13}C$ values from algae grown for 14 d in controlled conditions. This variation is attributable to an addition of inorganic nitrogen (and phosphate) and an interaction with light levels. We note that this range of variation in $\delta^{13}C$ values measured across specimens of *Ulva pertusa* spans much of the natural range of $\delta^{13}C$ values published for a number of different species of macroalgae (Raven et al. 2002). In other words, environmental variation (in this case due to experimentally induced light and nutrient regimes that were selected based on natural variation that we observed in field settings; Dudley 2007) was sufficient to make *U. pertusa* 'look' like any number of different primary producers. Understanding how the isotope signatures of primary producers vary across environmental gradients is therefore likely to be important to some applications of stable isotopes, e.g. those that use carbon isotope ratios to infer food sources of marine consumers (Dunton & Schell 1987, Dunton et al. 1989, Deegan & Garritt 1997, Wing et al. 2008). The effect of light availability on $\delta^{13}C$ values is likely to be due in part to the amounts of HCO_3^- and $CO_{2\,(aq)}$ used to meet the carbon demands of photosynthesis. Fractionation in the equilibrium reaction between HCO_3^- and CO_2 in seawater results in $\delta^{13}C$ values of $CO_{2\,(aq)}$ being approximately 10‰ lower than HCO_3^- (Zhang et al. 1995). The tissues of those species of algae that are able to utilise HCO_3^- tend to become enriched in ^{13}C under high light conditions as more energy is available for the active uptake of HCO_3^- (Kubler & Raven 1995, Cornelisen et al. 2007). Under conditions of high light and nutrient availability, increasing photosynthetic demand may also reduce efflux of ^{13}C-enriched inorganic carbon from cells, further increasing the $\delta^{13}C$ values of algal tissue (Sharkey & Berry 1986, Laws et al. 1997). When *U. pertusa* were supplied with sufficient N and P for growth, the differences in $\delta^{13}C$ values between light and shade treatments (3.5‰) were similar to the results of *in situ* work by Cornelisen et al. (2007), who showed an increase of about 3.7‰ in *U. pertusa* $\delta^{13}C$ values in a New Zealand fjord as irradiance increased from 200 to 2000 µE m^{-2} d^{-1}. In low nutrient treatments in our study, however, higher irradiance resulted in lower tissue-$\delta^{13}C$. This effect has previously been shown in N-limited microalgae (Riebesell et al. 2000). The low N:P ratio of ambient seawater during these experiments (~7.5:1) suggests that the algae that were not supplemented with nutrients were N limited rather than P limited (Atkinson & Smith 1983), and this is consistent with the commonly held theory that macroalgae in temperate regions are predominantly limited by the availability of N (Hanisak 1983, Smith 1984). N limitation is likely to result in reduced protein synthesis, potentially reducing the generation of transport proteins for HCO_3^- uptake, and therefore increasing the proportion of carbon that is taken up as (isotopically lighter) $CO_{2\,(aq)}$. Furthermore, N limitation may also reduce the ability of cells to harvest light (Riebesell et al. 2000), reducing photosynthetic demand for carbon, and resulting in lower tissue $\delta^{13}C$ values.

Effects of nitrogen source and light regime on $\delta^{15}N$ signatures, tissue-N and growth

Our results indicate that light dependence of $^{14}N/^{15}N$ fractionation for *Ulva pertusa* may be considerably less than has frequently been demonstrated for microalgae (Wada & Hattori 1978, Needoba & Harrison 2004, Needoba et al. 2004). Incorporation of $\delta^{15}N$ ratios within microalgae from aquatic environments is known to vary, sometimes substantially, both among and within species (e.g. 1 to 20‰ in *Thalassiosira pseudonana*; Waser et al. 1998a,b), and as functions of temperature, light and nutrient availability and source (Wada & Hattori 1978, Waser et al. 1998a,b, 1999, Needoba et al. 2003, Needoba & Harrison 2004). Consequently, observed microalgal $\delta^{15}N$ values are not necessarily a simple reflection of $\delta^{15}N$ values found in a particular nitrogen source.

In Expt 2, there was no difference in mean growth rates between *Ulva pertusa* supplied with nitrate or ammonium, within light treatments (Fig. 2C), suggesting that there was no long-term cost of utilisation of nitrate, over ammonium, as a sole source of nitrogen. However, despite there being no difference in growth of *U. pertusa* between the 2 nitrogen sources, fractionation of nitrogen sourced from nitrate was lower than fractionation of N sourced from ammonium. Nitrate-treated *U. pertusa* took up ^{15}N in close to the same proportion in 'shaded' and 'ambient light' treatments despite an 81% difference in light levels sustained over 14 d. The comparatively low fractionation of nitrate-supplied algae in both shaded and full-light treatments may indicate that the mechanism of fractionation during growth on nitrate that has been suggested for phytoplankton (Needoba et al. 2004) is less applicable to macroalgae, or at least to *U. pertusa*. Notably, the mechanism suggested by Needoba et al. (2004) relies on the efflux of ^{15}N-enriched internal pools of unreduced nitrate back into the external medium. The capacity of *U. pertusa* to store nitrate in unreduced form (Naldi & Wheeler 1999) may result in low efflux of nitrate from *U. pertusa* tissue even when nitrogen is supplied in excess to growth requirements (likely the case for shaded nitrate treatments in our study). Because fractionation is likely to occur as a result of chemical

processes involved in nitrogen assimilation prior to protein synthesis (Needoba et al. 2004), it is also probable that at least some of the fractionation occurring during growth of *U. pertusa* on ammonium in low light conditions is the result of isotope effects during diffusion across the plasma membrane. This fractionation does not appear to be as great in the active transport of nitrate across the plasma membrane and its reduction to ammonium under the same environmental conditions. Fractionation during light-saturated growth in *U. pertusa* supplied with ammonium was positive, indicating that there was a either a discrimination against ^{14}N at uptake or that there was a loss of ^{14}N subsequent to uptake. The former situation seems very unlikely, since it is generally accepted that discrimination during biological or physical uptake processes occurs against the heavier ^{15}N isotope (Bedard-Haughn et al. 2003). It is possible that in ammonium-supplied *U. pertusa*, the effect of light saturation may have resulted in small changes in cellular pH and therefore the relative proportion of NH_3 and NH_4^+. In theory, in light-saturated *U. pertusa* a slight increase in pH could have led to efflux of NH_3, which in turn would favour the retention of ^{15}N resulting in the fractionation observed in this treatment. This positive discrimination for ^{15}N during assimilation of ammonium has previously been documented in studies conducted on microalgae (Wada & Hattori 1978, Waser et al. 1998b) and supports evidence for short-term trends in positive discrimination for ^{15}N in *U.* (previously *Enteromorpha*) *intestinalis* (Cohen & Fong 2005). Only net fractionation was measured in this study, and thus no measures of uptake and efflux are available. Further work is required to elucidate the relative contribution of physiological processes to the observed patterns of fractionation for nitrate and ammonium in this study.

Our results enabled us to attribute variability in $^{14}N/^{15}N$ fractionation to the form of inorganic nitrogen (either ammonium or nitrate), light availability and the interaction between these 2 environmental variables. We suggest that the fractionation responses we observed in *Ulva pertusa* in Expt 2 may also be towards the extremes of the natural range (because in a given treatment we used only one N source). *In situ* studies in natural waters spanning summer and winter (and hence differences in available light) have shown little or no seasonal variation in *U. pertusa* $\delta^{15}N$ values (Cornelisen et al. 2007, Barr 2007). Similarly, *in situ* work by Cornelisen et al. (2007) suggests $\delta^{15}N$ differences on the order of 1‰ in *U. pertusa* growing over a PAR gradient spanning 200 to 2000 µE m^{-2} d^{-1} where both nitrate and ammonium were almost certainly available for uptake. This range of light levels was greater than the difference between our light and shaded treatments, which gave average values (for both experiments) of 266 µE m^{-2} d^{-1} or 1398 µE m^{-2} d^{-1}, respectively (note that these light levels resulted in either light-limited or light-saturated growth). Fractionation differences in contrasting light conditions *in situ* shown by Cornelisen et al. (2007) and Barr (2007) were closer to the range that we saw in *U. pertusa* supplied with nitrate (0.8‰) than with ammonium (3.7‰), and this may reflect the relative dominance of nitrate in coastal seawater. Ammonium concentrations in natural seawater typically comprise ~20% of DIN, and nitrate comprises ~75% (Sharp 1983). Notably, DIN concentrations in the open coastal sites of Barr (2007) were lower than the 10 µM used in this study, and previous research on higher plants, microalgae and bacteria has shown that fractionation increases with N availability (Wada & Hattori 1978, Hoch et al. 1992, McKee et al. 2002). It is possible that under conditions where N availability is low, fractionation may be low and less prone to change across light gradients. Further long-term studies using gradients of DIN concentrations are required to assess the effect of DIN availability on $\delta^{15}N$ values in macroalgae.

The use of a flow-through seawater system in our study facilitated the healthy growth of *Ulva pertusa* over several weeks and allowed constant addition of nitrogen at ecologically relevant concentrations. This method does carry the assumption that any natural seawater nitrogen that was not removed by the algal scrubber had a negligible influence on $\delta^{15}N$ of *U. pertusa* tissue. However, the algal nutrient scrubber removed most (60%) of the nitrogen present in seawater (which at Leigh Marine Laboratory typically has a low DIN concentration around 1 to 2 µM over the summer). As a corollary, the remaining (unstripped) inorganic nitrogen concentrations were on the order of 0.3 µM and 0.2 µM for ammonium and nitrate, respectively. Therefore, the subsequent addition of 10 µM nitrogen meant that approximately 95% of nitrogen available to *Ulva* was from the synthetic source. Using a simple 2-end mixing calculation (e.g. Spies et al. 1989), the concentrations of natural seawater measured (assuming a $\delta^{15}N$ value for seawater DIN of 6 to 8‰; Miyaki & Wada 1967, Wada et al. 1975, Sigman et al. 1997, 2000) could generate differences in $\delta^{15}N$ values of up to 0.24‰ between light and shade ammonium treatments and 0.11‰ for nitrate treatments.

SUMMARY

Our experiments demonstrate systematic variation in $\delta^{15}N$ and $\delta^{13}C$ signatures arising from interactions between light intensity and inorganic nitrogen source. We conclude that while $\delta^{13}C$ in *Ulva pertusa* may vary substantially depending on nutrient status and light

availability, $\delta^{15}N$ in *U. pertusa* more closely reflects the nitrogen source pool in seawater. We suggest that the comparatively small range of $\delta^{15}N$ values expressed in *Ulva* supplied with nitrate (0.8‰), despite contrasting light levels, indicates that $\delta^{15}N$ in *U. pertusa* might represent a good proxy for $\delta^{15}N$ of DIN in nitrate-dominated coastal seawater.

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