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Stable isotope (^{13}C) and O_2 micro-optode alternatives for measuring photosythesis in seaweeds

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ABSTRACT: Many photosynthetic studies with macroalgae are based on oxygen evolution. This approach is preferred over ^{14}C tracer techniques since oxygen electrodes are generally inexpensive and radioactive wastes are not produced. Nevertheless, oxygen techniques do not directly measure carbon assimilation; thus, primary production estimates rely on the conversion of oxygen units to carbon units via a photosynthetic quotient, which may vary depending on physiological state and nutrient status of the alga. We present in detail 2 methods for measuring photosynthesis in macroalgae, both of which should enhance prospects for photosynthetic research, particularly under field conditions. First, a carbon uptake procedure is described which relies on incorporation of a stable ^{13}C isotope label. Important advantages of the ^{13}C method include simplicity of sample processing, avoidance of environmental hazards and restrictions of radioactive ^{14}C, and ability to be used as a dual tracer with ^{15}N. Second, we employed a fiber-optic micro-optode system for measurement of dissolved oxygen. The fluorescence-based optodes stabilize quickly (<15 s), do not consume oxygen, and are simple to set up and maintain. Oxygen and carbon photosynthesis were assessed concurrently in a light/dark chamber design. Both techniques resolved significant decreases in light-saturated photosynthesis (P_{max}) in the kelp *Laminaria hyperborea* collected subtidally at 10 m compared to kelp at 0 m (from mean low tide). Oxygen and carbon photosynthetic rates agreed well with published values and were mutually consistent, considering a reasonable photosynthetic quotient. Estimated measurement errors (<2%) associated with the ^{13}C technique were less than intrinsic variation (>20%) in photosynthetic rates between kelp individuals with similar light histories. Both techniques should provide an alternative to traditional photosynthetic protocols and stimulate further primary production research in macrophytes.

KEY WORDS: ^{13}C carbon fixation · *Laminaria hyperborea* · Oxygen evolution · Optodes · Photosynthesis · Stable isotopes

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INTRODUCTION

Photosynthetic conversion of sunlight provides basic organic compounds for energy and matter flow through most aquatic ecosystems. During photosynthesis, inorganic carbon is reduced to simple carbohydrate(s), and O_2 is liberated as a by-product. Therefore, the most fundamental measurements of photosynthesis and plant/algal productivity involve assessing either carbon uptake or oxygen evolution over a given period of time. As an alternative, pulse-amplitude-modulated (PAM) chlorophyll fluorescence was enthusiastically explored as a rapid and non-intrusive primary production measurement (Beer et al. 1998, 2000, Figueroa et al. 2003, Beer & Axelsson 2004), but the relationship between production rates and PAM-derived electron transport rates becomes increasingly uncertain at high irradiances or in algae with thick thalli (Beer & Björk 2000, Franklin & Badger 2001, Longstaff et al. 2002, Perkins et al. 2002). Methods of measuring photosynthetic gas exchange, despite their underlying assumptions and associated chamber effects, remain neces-

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sary in many primary production studies (Binzer & Middelboe 2005, Choo et al. 2005).

Since its introduction by Steemann-Nielsen (1952), radioactive carbon (^{14}C) as a tracer of photosynthesis has become the standard in phytoplankton primary production research (Geider & Osborne 1992, Falkowski & Raven 1997). In contrast, macroalgal researchers frequently employ oxygen evolution procedures in production experiments (Gerard 1988, Geider & Osborne 1992, Hatcher et al. 1997, Aguilera et al. 1999), with ^{14}C-based field experiments relatively rare (Berger & Bate 1986, Dunton & Schell 1986, Dunton & Jodwalis 1988). The predominate use of oxygen procedures is ostensibly due to low cost and simplicity as compared to macrophyte ^{14}C protocols. While ^{14}C-labeled phytoplankton can be filtered and dissolved directly into liquid scintillation solution, macrophyte tissue must be either dry combusted (Berger & Bate 1986, Dunton & Schell 1986) or solubilized (Lewis et al. 1982, Arnold & Littler 1985) prior to ^{14}C counts. Macrophyte experiments generate large volumes of radioactively labeled seawater, which accrue additional costs in storage, shipping, and disposal. Moreover, oxygen evolution measurements tend to overestimate primary productivity because other metabolic processes (e.g. nitrate/nitrite reduction) compete with carbon fixation for reducing power and ATP energy generated during photosynthetic light reactions. Ultimately, carbon is the energy currency moved through trophic systems; therefore, direct determination of photosynthetic carbon fixation is preferential when constructing macroalgal carbon budgets or addressing ecosystem-level primary production questions.

We present an alternative method to ^{14}C tracer experiments, which uses the stable isotope of carbon (^{13}C) as a label of photosynthetically incorporated carbon within macroalgae. Slawyk et al. (1977, 1979) originally presented a ^{13}C procedure to measure photosynthetic production in phytoplankton, and Hama et al. (1983, 1993), Mousseau et al. (1995) and Genkai-Kato et al. (2003) further developed the technique, also for phytoplankton studies. A known amount of ^{13}C-labeled sodium (bi)carbonate is added to seawater, and atom percent ^{13}C (at.% ^{13}C) in phytoplankton cells is determined after a light trial, i.e. a certain light level for a defined length of time. Photosynthetic rate is then proportional to the mass balance between: (1) at.% ^{13}C of cells before and after the incubation, (2) at.% ^{13}C of the labeled dissolved inorganic carbon (ΣCO_2) source, and (3) concentration of particulate organic carbon either before (sensu Slawyk et al. 1977) or after (sensu Hama et al. 1983) the incubation. Mateo et al. (2001) more recently introduced the ^{13}C method to seagrass studies and showed good consistency between ^{13}C and both ^{14}C and O_2 production rates.

The present study demonstrates the utility of the ^{13}C method in macroalgal research by comparing light-saturated photosynthetic rates of the kelp *Laminaria hyperborea* (Gunn.) Foslie collected from 2 depth intervals. We revisit the derivation of the ^{13}C rate equation to highlight assumptions, suggest minor analytical improvements, and present adaptations to macroalgal applications. We also describe a straightforward method to determine inorganic $\delta^{13}C$ in labeled seawater. Photosynthetic rate is dependent on the amount of inorganic ^{13}C enrichment, and analytical determination of this value is critical if any pre-incubation manipulations alter ^{13}C relative to total inorganic carbon.

We also introduce fiber-optic oxygen micro-sensors (micro-optodes) to measure photosynthetic oxygen evolution. Oxygen optodes utilize the fluorescence quenching ability of oxygen to relate oxygen concentration in seawater to fluorescence quantum yield of an immobilized fluorophore in the probe (Klimant et al. 1995). The Microx TX oxygen meter and associated micro-optodes (PreSens) have promise in both field and laboratory applications (Glud et al. 1999, 2000). The instrument is compact and lightweight and is controlled from a laptop computer. In our configuration, the micro-optode fiber-optic is housed inside a syringe needle, which allows easy insertion into sealed chambers for either real-time oxygen measurements or before and after measurements in classic light/dark bottle incubations. Compared to Clark-type oxygen (macro)electrodes, optodes have a rapid response time, and, since they do not consume oxygen, they do not require correction for consumption effects.

MATERIALS AND METHODS

Kelp collection and tissue preparation. Photosynthetic experiments were conducted in April 2001 in a field laboratory on the island of Finnøy, Norway. Carbon uptake and oxygen evolution were compared in the kelp *Laminaria hyperborea*, collected near the sea surface (0 m relative to mean low water level) and at 10 m. Adult kelp were harvested 1 d prior to the experiments, and whole kelp thalli were stored overnight, underwater, in dark collection bags suspended in a protected boat channel. *In situ* water temperatures at this time were 3 to 5°C with 32‰ salinity, and peak sunlight was about 1400 µmol photons m^{-2} s^{-1} at 0 m and 200 µmol photons m^{-2} s^{-1} at 10 m.

A 1.8 cm diameter brass coring device was used to excise kelp discs from the first lamina, i.e. the newly formed photosynthetic material produced that winter/spring. All discs were cut from tissue at least 10 cm from the basal, meristematic region of the lamina, and tissue discs were kept under dim laboratory light in a

temperature-controlled (5°C) seawater container. Mucilage was produced initially from the margins of the kelp discs, but after 1 h the wound response had ceased. Photosynthetic rates were determined in 10 separate photosynthesis versus irradiance experiments, with 5 individual kelp plants from 2 depths: 0 and 10 m.

Incubation medium. Seawater used in photosynthetic incubations was both enriched in inorganic ^{13}C and partially deoxygenated. Several days before light experiments, $NaH^{13}CO_3$ (99% ^{13}C, Cambridge Isotope Laboratory) was added to 0.45 µm filtered seawater in a large-volume carboy. The carboy lid was left loose, and the container was occasionally shaken to allow ΣCO_2 to exchange with the atmosphere and to partially re-equilibrate. Prior to incubation, a smaller volume of ^{13}C-enriched seawater was brought to experimental temperature (5°C), and O_2 was stripped from solution by bubbling with N_2 for 8 min. As N_2 also displaced ΣCO_2, longer bubbling times decreased photosynthetic rates presumably due to carbon limitation. Stripping resulted in initial experimental oxygen concentrations of ca. 20 to 30% seawater saturation and prevented super-saturation degassing of O_2 during incubations (Table 1).

For short-duration experiments (1 to 2 h), significant ^{13}C enrichment was needed to generate a measurable ^{13}C uptake signal in kelp tissue (Table 1). From the amount of $NaH^{13}CO_3$ mixed with the volume of seawater in the carboy, at.% ^{13}C was calculated to increase from ca. 1% ^{13}C in natural seawater to 45% for Light Trials 1 to 3 (i.e. 2.908 g $NaH^{13}CO_3$ in 20 l seawater) and 50% for Trials 4 to 10 (i.e. 4.000 g $NaH^{13}CO_3$ in 22 l seawater). Addition of this amount of labeled bicarbonate nearly doubled ΣCO_2 concentration initially, but by allowing the incubation medium to re-equilibrate with the atmosphere for several days and by bubbling seawater with $N_{2(g)}$ prior to incubation, ΣCO_2 concentrations at the beginning of light experiments were reduced to just 13% (mean, Trials 1 to 3) and 17% (mean, Trials 4 to 10) greater than *in situ* concentrations (Table 1).

Water samples for ΣCO_2 and at.% ^{13}C analysis were taken prior to each incubation. Both *in situ* seawater and pre-trial media samples were collected in 60 ml serum bottles (Wheaton). Bottles were stoppered with butyl-rubber septa and aluminum caps so that air bubbles were not trapped inside. Water samples were fixed by injecting 0.2 ml saturated $HgCl_2$ into serum bottles, while an equal volume of seawater was removed. Bottle tops were sealed with parafilm to ensure minimal evaporation losses.

Photosynthesis versus irradiance (*P*/*I*) experiments. Photosynthetic rates were determined for *Laminaria hyperborea* in a spectral incubator, the photoinhibitron (Neale & Fritz 2002), which was modified from phytoplankton applications to be used with kelp discs. The apparatus consists of: (1) a black, anodized, aluminum block bored to hold 80 quartz-bottomed incubation cuvettes (20 ml), (2) a continuous-flow water bath to control temperature, (3) a 2500 W xenon lamp light source, and (4) a central mirror that reflects light up through the apparatus. The spectral composition and intensity of irradiance treatments were manipulated with Schott polychromatic cutoff filters and neutral density screens. In the macroalgal configuration, photosynthesis rates were determined in 48 different light treatments in each 1.5 h experimental trial, though only photosynthesis determined using 395 nm cutoff filter is presented here. Neutral density interference filters produced 6 different irradiances under the 395 nm filter, which was effectively a photosynthetically active radiation (PAR)-only spectral treatment. Prior to each *P*/*I* trial PAR irradiances were confirmed

Table 1. ΣCO_2 and dissolved O_2 (DO) in natural *in situ* seawater and in experimental seawater at the beginning of each complete photosynthesis versus irradiance replicate experiment. Values are reported as means ± SE with sample number in parentheses (n) in column headings. ND: no data

Trial	Depth (m)	*In situ* dissolved gases[a] ΣCO_2 (4) (mM)	at.% ^{13}C (5)	DO (3) (µM)	Experimental dissolved gases ΣCO_2 (2) (mM)	at.% ^{13}C (2)	DO (9) (µM)
1	0	2.13 ± 0.01	1.1108 ± 0.0002	379 ± 2	2.40 ± 0.00	64.159 ± 0.286	115 ± 2
2				393 ± 1	2.45 ± 0.02	59.656 ± 0.089	139 ± 6
3				373 ± 1	2.37 ± 0.00	63.732 ± ND	103 ± 2
4				372 ± 3	2.44 ± 0.03	80.800 ± 0.512	80 ± 3
5				377 ± 1	2.49 ± 0.01	80.323 ± 0.415	71 ± 2
6	10	2.13 ± 0.01	1.1108 ± 0.0002	373 ± 0	2.46 ± 0.02	79.095 ± 0.127	92 ± 1
7				373 ± 0	2.51 ± 0.03	79.154 ± 0.154	76 ± 3
8				381 ± 1	2.47 ± 0.02	78.409 ± 2.417	65 ± 2
9				384 ± 1	2.49 ± 0.01	78.411 ± 0.574	90 ± 2
10				393 ± 1	2.51 ± 0.04	78.459 ± 0.117	84 ± 4

[a]Values of at.% ^{13}C for seawater off the island of Finnøy were determined for water samples collected in September 1999

with a spherical quantum sensor (Biospherical Instruments QSL-100), and 6 light treatments averaged 20, 248, 668, 702, 996, and 1204 µmol photons m^{-2} s^{-1} for the 10 *P*/*I* trials. Temperature was maintained at 5 ± 1°C by adding ice as needed to a large volume (ca. 50 l) reservoir and by continuously cycling this water through the photoinhibitron's water bath.

Incubation cuvettes were filled with ^{13}C-enriched and deoxygenated seawater, and kelp discs were suspended in cuvettes about 1 cm from the cuvette bottom (Fig. 1). A rubber o-ring, crosshatched with thin transparent thread, supported the disc. Suspending the disc in the cuvette assured proper orientation perpendicular to the light source and promoted unimpeded water movement around the thallus. After removing air bubbles, cuvettes were sealed with modified silicon stoppers.

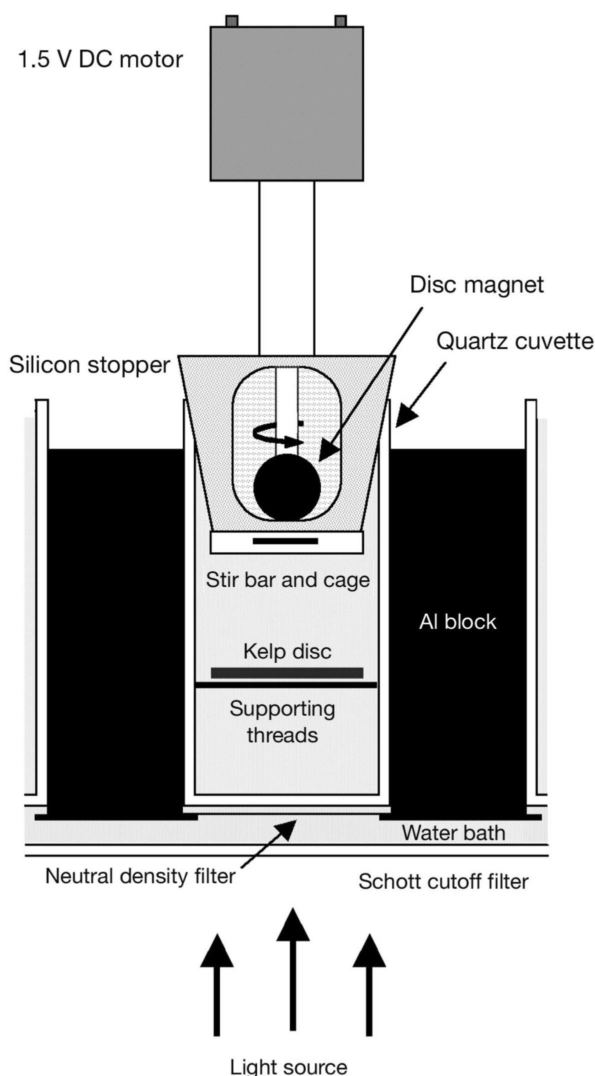

Fig. 1. Cross-section of incubation cuvette and motorized stirring mechanism in photoinhibitron. Light originates from a 2500 W xenon lamp and passes though a Schott cutoff filter (395 nm), UV-transparent Plexiglas (bottom of water bath), a neutral density screen, and the quartz-bottomed cuvette. Magnetic stir bar continuously mixes water inside cuvette, discouraging diffusive boundary layers and promoting oxygen and carbon exchange between photosynthetic tissue and seawater medium. Temperature is maintained with a continuous-flow water bath

Vigorous water motion was required in each cuvette to discourage formation of boundary layers and decreased carbon diffusion rates to the thallus. Silicon stoppers were fitted with motorized magnetic stir bars (Fig. 1). A strong disc magnet was attached via a short axle to a 1.5 V DC electric motor (Radio Shack). The device was then secured inside a bored-out silicon stopper, and the stopper was sealed at the bottom with a plastic cage that housed a small magnetic stir bar. Once cuvettes with motors were set into the photoinhibitron, motors were connected to a B&K Precision 20 amp DC power source, and stir bar speeds were adjusted by controlling the voltage supplied to the motors.

In addition to 6 light treatments, each *P*/*I* trial consisted of 3 dark treatments and 3 oxygen 'blanks'. Oxygen blanks were deoxygenated seawater without kelp tissue, whereas dark treatments began with both ^{13}C-enriched and oxygen-saturated seawater. Discs incubated in opaque cuvettes allowed for quantification of both light-independent carbon fixation and respiration (O_2 consumption). Both dark treatments and blank cuvettes were placed in the photoinhibitron's water bath, but out of direct light.

Both oxygen and carbon rate equations presume that the rate of production is constant throughout the duration of the light treatment. Rate linearity was not confirmed experimentally for *Laminaria hyperborea* in the photoinhibitron set-up, but Mateo et al. (2001) demonstrated linearity in seagrasses in both ^{13}C uptake and O_2 evolution during incubation times shorter than 4 to 6 h. Nonetheless, this issue should be considered further as Macedo et al. (1998) have shown that P^B_{max} in phytoplankton is time-dependent.

Measurement and calculation of oxygen evolution. Carbon uptake and oxygen evolution were determined concurrently in each replicate *P*/*I* trial. Oxygen concentration in incubation seawater was measured before and after light/dark treatments. Initial subsamples of deoxygenated incubation medium were collected every time 6 sample cuvettes were placed in the photoinhibitron. Subsamples were covered with motorized stoppers, and magnetic stir bars in cuvettes briefly activated to homogenize seawater. Stoppers were then removed, and oxygen concentrations determined immediately. A needle-type fiber-optic oxygen optode (PreSens, Model NTH operating range: 0 to 1.4 mmol, resolution: 2.83 µmol) and a temperature

probe were inserted 1.5 cm into seawater, and temperature-compensated O_2 concentration was monitored with a Microx TX (PreSens) oxygen meter coupled to a PC laptop computer. The instrument was controlled with proprietary software (Microx v.1.4.6). Oxygen measurements stabilized within 15 s and remained constant (±5 µM) for several minutes, which suggests minimal O_2 exchange with atmosphere during measurement. Micro-optodes were calibrated prior to each trial with oxygen-saturated seawater and with seawater depleted of O_2 by addition of $Na_2S_2O_4$.

After light/dark treatments, sample cuvettes were removed from the photoinhibitron and placed in a temperature-monitored water bath. O_2 concentrations were determined within 5 min of removal from the photoinhibitron. Following a brief pulse from the electric motor to homogenize seawater in cuvettes, kelp discs were carefully removed, and O_2 concentration was immediately analyzed. Seawater volume was measured for each cuvette, as this measure varied by as much as 20% between incubation cells.

Net oxygen evolution rates were calculated from the difference in final O_2 concentrations and (blank corrected) O_2 concentrations at the beginning of the experiment, divided by the time duration of the incubation. Dark respiration rates were added to the net O_2 evolution rates to yield gross photosynthesis. Though we recognize that respiration rates may increase during light treatments (Falkowski & Raven 1997), the experimental design prevented us from measuring dark respiration after light trials. We assumed that before- and after-treatment respiration rates were equivalent, and this assumption is supported in photosynthetic studies with *Laminaria saccharina* (Gerard 1988). Photosynthetic rates were normalized to thallus area (single-sided) with final units of µmol O_2 m^{-2} s^{-1}.

Kelp tissue carbon isotope measurement. Carbon uptake rates were based on the amount of ^{13}C incorporated by photosynthesis into tissue organic carbon during the time of incubation. Rates were obtained from measurement of natural abundance $\delta^{13}C$ in kelp organic matter and $\delta^{13}C$ of kelp after the experiment. Natural abundance $\delta^{13}C$ was determined with 3 replicate thallus samples from 4 individual kelp plants collected at either 0 or 10 m. Initial tissue samples were removed from the first lamina and immediately dried at 60°C. As $\delta^{13}C$ variation between individual kelp plants was <0.2% of the expected change in $\delta^{13}C$ at typical photosynthetic rates, initial experimental $\delta^{13}C$ values were considered equal to natural abundance $\delta^{13}C$ in kelp tissue at each collection depth. After incubation, kelp discs were thoroughly rinsed twice in seawater and once in freshwater, to remove excess bicarbonate label from the thallus. Discs were blotted dry, placed in micro-centrifuge tubes (for storage), and dried at 60°C. Dry weights of kelp discs were recorded, and discs were ground to a fine powder for stable carbon isotope analysis.

Isotopic composition of both natural abundance and experimental kelp discs were analyzed using a Finnigan MAT DeltaPlus continuous flow stable isotope mass spectrometer attached to a Carlo Erba NC 2500 elemental analyzer (EA-IRMS). The instrument was calibrated daily using a secondary standard (powdered chitin from shrimp shells, Sigma Aldrich, No. C-8909) and related to $\delta^{13}C$ of Pee Dee Belemnite. Mean chitin $\delta^{13}C$ was –19.91‰, with a precision of ±0.10 CV (coefficient of variation or percent standard deviation of mean), mean (±CV) chitin percent carbon was 44.74 ± 0.64%.

Experimental kelp $\delta^{13}C$ values ranged from 100 to 300‰ at typical photosynthetic rates, and these artificially heavy samples suffered a systematic underestimation when analyzed with an instrument calibrated against a light $\delta^{13}C$ standard. Presumably, this is a result of less efficient ionization of $^{13}CO_2$ (i.e. instrument fractionation) at high $^{13}CO_2$ concentrations. To correct for this bias, a calibration curve for heavy $\delta^{13}C$ values was produced using various $\delta^{13}C$ $NaHCO_3$ standards (Fig. 2). Seven heavy $\delta^{13}C$ standards were created by mixing measured quantities of laboratory-grade $NaHCO_3$ (EM Science, No. SX0320-1) with $NaH^{13}CO_3$ to produce expected values of $\delta^{13}C$ between –5.93‰ ($NaHCO_3$ only) and 1000‰. Mixed sodium bicarbonate isotope standards were dissolved in 200 ml deionized water, and solutions were shaken vigorously to homogenize dissolved ions. Each standard was then freeze-dried, and the resulting solid ground to a fine powder. Heavy standards were analyzed on the EA-IRMS, and the relationship between observed and expected $\delta^{13}C$ was linear over the $\delta^{13}C$ range measured. Experimental kelp samples were then corrected by the best-fit linear equation.

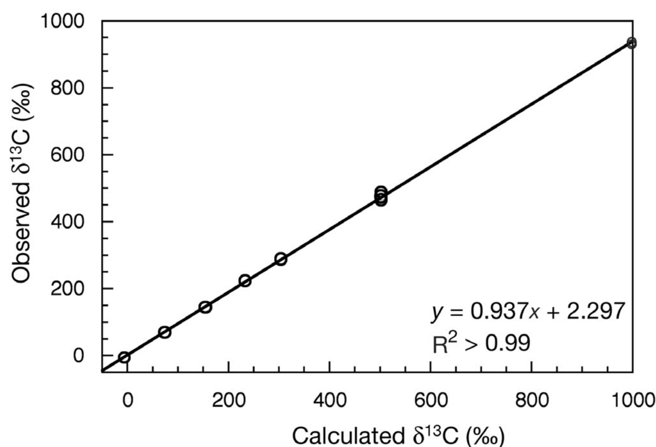

Fig. 2. $\delta^{13}C$ calibration curve for thallus isotope samples with artificially heavy isotopic composition (n = 3)

Incubation medium carbon isotope measurement. Short-duration ^{13}C *P/I* experiments require a heavily enriched ^{13}C source to obtain measurable $\delta^{13}C$ changes in samples. In this experiment, seawater was initially enriched 50% in ^{13}C, but after O_2 stripping was 60 to 80% enriched, implying N_2 bubbling favored $^{12}CO_2$ release (Table 1). Photosynthetic rate calculation depends critically on at.% ^{13}C of the inorganic carbon source, so the $\delta^{13}C$ of enriched seawater used in incubations was determined analytically using general concepts and procedures described in Miyajima et al. (1995).

All water samples for isotopic analysis were stored in 60 ml Wheaton glass serum bottles (actual inner volume without headspace 70.5 ± 0.5 ml). At ambient laboratory temperature (23 to 25°C) and atmospheric pressure (1 atm, sea level), a 5 ml gas headspace was created by injecting ultra-pure $He_{(g)}$ with a 5 ml gas-tight syringe (Hamilton) into each serum bottle. An equal amount of fluid was removed from each bottle to equalize pressure. Samples were then acidified with addition of 0.50 ml of 6.0 N HCl, and again an equal volume of solution was removed. The HCl solution had been previously stripped of dissolved ΣCO_2 by bubbling with the same He above for a minimum of 3 h. Acidification effectively converted inorganic carbon forms of HCO_3^- and CO_3^{-2} into aqueous CO_2. Sample bottles were stored upside-down in the dark for at least 40 h to allow for equilibration of $CO_{2(aq)}$ with headspace $CO_{2(g)}$ (Miyajima et al. 1995).

After equilibration, 1 ml of headspace gas was injected with a 1 ml gas-tight syringe (Hamilton) into the gas-injection port between the oxidation and reduction columns of the Carlo Erba EA. Before gas samples were taken from bottle headspace, the syringe was purged several times with He to expel residual sample/atmospheric gases trapped in the needle. Even though combustion was not necessary with this procedure, EA furnaces were kept at operating temperatures to maintain stable analytical conditions. $\delta^{13}C$ of each injected sample was determined with the IRMS.

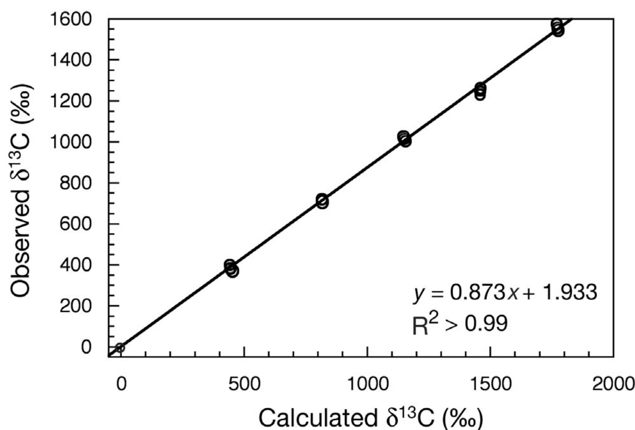

Fig. 3. Dilution $\delta^{13}C$ calibration curve for ^{13}C-enriched seawater media (n = 3)

If inorganic ^{13}C was increased by 50% in seawater, $\delta^{13}C$ values would approximately equal 88 000‰. Extremely heavy $\delta^{13}C$ exceeds the analytical range of the IRMS configured for natural abundance measurements. Specifically, maximum amplifier output voltage on the mass 45 Faraday collection cup saturates with the abundance of $^{13}CO_2^-$ ions generated using this procedure. Instead of replacing the internal high ohmic feedback resistor in the voltage amplifier, we chose to dilute the $\delta^{13}C$ value of our enriched seawater samples. First, a calibration curve was produced for dilute $\delta^{13}C$ samples (Fig. 3). Five standard solutions of $\delta^{13}C$ equal to 33 200, 61 800, 85 200, 107 700, and 130 200‰ were created with seawater (1.64‰, 2.33 mM natural abundance ΣCO_2) and $NaH^{13}CO_3$. In glass serum bottles, each $\delta^{13}C$ stock was then diluted 1:69.5 (v/v) in 2.25 mM $NaHCO_3$ (–5.93‰). A $NaHCO_3$ solution without enrichment was included as the sixth standard in the curve. Bottles were sealed, and isotopic compositions of diluted standards were determined on the EA-IRMS as described above. Dilution series $\delta^{13}C$ values observed after EA-IRMS analysis systematically under-represented $\delta^{13}C$ values expected from quantities of $H^{12}CO_3^-$ and $H^{13}CO_3^-$ mixed, but this bias was linear over the range of standards created (Fig. 3).

Enriched seawater samples for each photosynthetic trial were prepared and analyzed similarly to the dilution standards. After subsampling 1 ml for $\delta^{13}C$ composition, ΣCO_2 of the remaining seawater was determined on an IO Analytical Wet Oxidation TOC Analyzer (Model 1010). Observed $\delta^{13}C$ of diluted samples were adjusted to calculated diluted seawater $\delta^{13}C$ with the linear calibration equation. Converting observed $\delta^{13}C$ to calculated $\delta^{13}C$ in enriched media samples also corrected for (1) equilibrium isotopic discrimination between $CO_{2(aq)}$ in the bottle and $CO_{2(g)}$ in the headspace and (2) any chemical interference associated with salinity (ionic strength) or with the presence of volatile organic compounds. Final $\delta^{13}C$ values of enriched seawater samples were calculated from the diluted $\delta^{13}C$ values and mass balance equations below.

Calculation of ^{13}C photosynthetic rate. Photosynthetic rate was calculated from the isotopic mass balance of tissue organic carbon and seawater dissolved inorganic carbon (ΣCO_2), determined both before and after light treatment. The mass balance approach followed Hama et al. (1983, 1993) and Mateo et al. (2001), with analytical modifications for macrophytes. $\delta^{13}C$ was measured for both photosynthetic samples and incubation seawater. The ratio of $^{13}C/^{12}C$ for each sample (R_{sam}) is calculated from the definition of $\delta^{13}C$ such that:

$$\delta^{13}C = \left(\frac{R_{sam}}{R_{PDB}} - 1\right) \times 1000 \quad (1)$$

where R_{PDB} is the Pee Dee Belemnite carbon isotope standard (R_{PDB} = 0.0112372). at.% ^{13}C is calculated from $R_{sam}/(R_{sam} + 1) \times 100$ and is equivalent to the mole ratio of ^{13}C to total carbon ($^{12}C + {}^{13}C$) in the sample.

at.% ^{13}C in $H^{13}CO_3$-enriched incubation seawater (a_{se}) is derived from the natural abundance of ^{13}C in seawater and the amount of ^{13}C bicarbonate label added. When enriched seawater is used immediately after the addition of ^{13}C bicarbonate, then a_{se} can be calculated from the mass balance such that:

$$a_{se}C_{se} = a_{sn}C_{sn} + a_{13b}C_{13b} \quad (2)$$

where a_{sn} is at.% ^{13}C in natural seawater before enrichment, a_{13b} is at.% ^{13}C in $NaH^{13}CO_3$ added (= 99%), C_{13b} is mmol ^{13}C bicarbonate added, C_{sn} is mmol ΣCO_2 in natural seawater, and C_{se} is total mmol of ΣCO_2 in enriched seawater at the beginning of the experiment ($C_{se} = C_{sn} + C_{13b}$). C_{sn} and a_{sn} can be determined experimentally as described below, or C_{sn} can be approximated from salinity (Parsons et al. 1984) and a_{sn}, assumed equal to 1.1112% (= $\delta^{13}C$ of 0.0‰; Mateo et al. 2001).

If it is suspected that the ratio of ^{13}C to ^{12}C in enriched seawater has changed prior to the experiment, then a_{se} must be measured analytically. Addition of ^{13}C bicarbonate to seawater results in high $\delta^{13}C$ values that must be diluted before analysis on the EA-IRMS as noted previously. Once the diluted seawater $\delta^{13}C$ value has been determined (see 'Incubation medium carbon isotope measurement' above), a_{se} can be calculated from the mass balance such that:

$$a_{sd}C_{sd} = a_{se}C_{se} + a_{12b}C_{12b} \quad (3)$$

where a_{sd} is at.% ^{13}C of diluted incubation seawater, a_{12b} is at.% ^{13}C of stock $NaH^{12}CO_3$, C_{sd} is total mmol ΣCO_2 in diluted seawater, and C_{12b} is mmol of unlabeled bicarbonate in dilution stock. As $C_{sd} = C_{se} + C_{12b}$, then:

$$a_{se} = a_{sd} + DIC_{12b}V_{12b}\left(\frac{a_{sd} - a_{12b}}{DIC_{se}V_{se}}\right) \quad (4)$$

in terms of ΣCO_2 concentration (mM) of stock solution (DIC_{12b}) and total ΣCO_2 concentration in incubation seawater (DIC_{se}). V_{se} is the volume (l) of DIC_{se} added to the serum bottle, and V_{12b} is the volume of dilution stock in the serum bottle (i.e. V_{12b} = volume serum bottle $-V_{se}$ = 0.0695 l).

Once a_{se} is known, the isotope mass balance equation for the derivation of photosynthetic rate begins as:

$$a_{tn}C_{tn} + a_{se}C_{se} = a_{tf}C_{tf} + a_{sf}C_{sf} \quad (5)$$

where a_{tn} is the at.% ^{13}C in initial tissue (i.e. natural abundance of ^{13}C to total C in the alga), a_{tf} is the at.% ^{13}C in the final tissue after incubation, a_{sf} is the at.% ^{13}C remaining in the seawater after incubation, C_{tn} is the number of mmol organic carbon in the algal tissue, C_{tf} is the number of mmol carbon in the final algal tissue, and C_{sf} is the number of mmol ΣCO_2 remaining in the seawater after incubation. The change in tissue carbon over time (ΔC) is the difference between C_{tf} and C_{tn}. If $C_{tn} = C_{tf} - \Delta C$ and $C_{sf} = C_{se} - \Delta C$ are substituted into the mass balance Eq. (5) and if it is assumed that $a_{sf} = a_{se}$, then the equation can be rewritten as:

$$a_{tf}C_{tf} = a_{tn}(C_{tf} - \Delta C) + a_{se}\,\Delta C \quad (6)$$

The assumption that at.% ^{13}C of enriched seawater medium (a_{se}) does not change over the incubation period allows photosynthetic rate calculation to be simplified and eliminates the need to determine $\delta^{13}C$ of media after incubation. With a photosynthetic rate typical of shallow-water kelp and with an algal tissue to container volume ratio equal to our experiment with the photoinhibitron, after 1 yr in continuous light a_{sf} would remain 99% of the initial value of a_{se}.

Photosynthetic rate (P, µmol C mg dry wt^{-1} h^{-1}) is derived from Eq. (6) to yield carbon uptake (ΔC) in terms of at.% ^{13}C of initial and final algal tissues, at.% ^{13}C of enriched seawater, and the carbon content in the algal sample after incubation:

$$P = \frac{a_{tf} - a_{tn}}{a_{se} - a_{tn}} \times \frac{C_{tf}}{t} \times 83.2\alpha \quad (7)$$

Algal carbon (i.e. C_{tf}) content on a dry weight basis is determined from mass spectrometer (or elemental analyzer) analysis. If photosynthesis is normalized to dry weight, then C_{tf} is simply tissue weight percent carbon (%C). To normalize carbon uptake on a single-sided areal basis, we multiplied C_{tf} by the dry weight to area ratio particular to each individual sample. Thus, our photosynthetic rates are expressed in µmol C m^{-2} s^{-1}.

Eq. (7) includes a fractionation correction, α, that should be evaluated on a study-by-study basis. Enzyme kinetics of RUBISCO lead to a temperature-dependent discrimination against the heavier ^{13}C isotope relative to ^{12}C (O'Leary 1988). Procedurally, this isotope discrimination results in a slight bias (1 to 3%) of apparent carbon fixation rate. To compensate, P can be corrected by a kinetic fractionation factor (α). Depending on the study, α can be chosen to be an average fractionation of 1.025 (Hama et al. 1983), an algal-specific fractionation (present study), or omitted entirely from the calculation (Slawyk et al. 1977, Mousseau et al. 1995, Mateo et al. 2001). An algal-specific fractionation factor can be estimated from the natural *in situ* $\delta^{13}C$ of ΣCO_2 ($\delta^{13}C_{sn}$) and algal tissue ($\delta^{13}C_{tn}$), specifically:

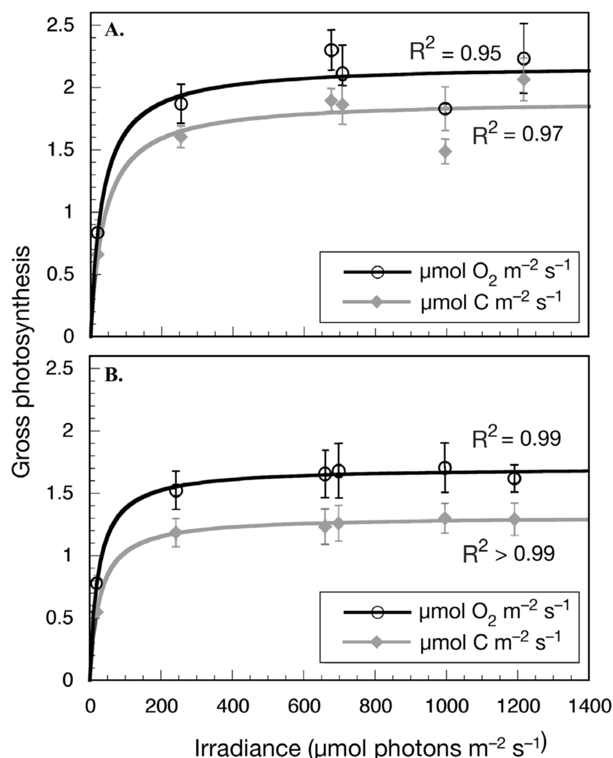

Fig. 4. *Laminaria hyperborea*. Gross photosynthesis versus irradiance responses for kelp collected from: (A) 0 m and (B) 10 m depth. Oxygen evolution and ^{13}C carbon fixation were measured concurrently for each light treatment. Individual data points are means ± SE, n = 5), and the Michaelis-Menten formulation was fit to mean data points

$$\alpha = \frac{\delta^{13}C_{sn} - \delta^{13}C_{tn}}{1000 + \delta^{13}C_{tn}} + 1 \quad (8)$$

A second correction can be included in P calculations to compensate for the amount of organic carbon fixed by β-carboxylation, i.e. light-independent carbon fixation (Johnston & Raven 1986, Cabello-Pasini & Alberte 1997). Non-photosynthetic carbon fixation is determined from carbon uptake in dark incubation cuvettes (a_{td}) run concurrently with light trials, and rate of β-carboxylation is calculated similarly to P in Eq. (7). Alternatively, calculating ΔC using dark trials as initial at.% ^{13}C values would also compensate for β-carboxylation. In our case, dark β-carboxylation rates could not be resolved because dark trial at.% ^{13}C tissues were not significantly different than natural abundance at.% ^{13}C (a_{tn}(0 m) = 1.0922, a_{td}(0 m) = 1.0913; t-test: p = 0.11 and a_{tn}(10 m) = 1.0896, a_{td}(10 m) = 1.0907; t-test: p = 0.10). If β-carboxylation is a significant fraction of carbon fixed, it should be noted that enzymes involved with β-carboxylation may have a different α fractionation factor than RUBISCO (Arnelle & O'Leary 1992).

RESULTS

Both oxygen- and carbon-based measurements of photosynthesis yielded significant differences in maximum photosynthesis (P_{max}) between *Laminaria hyperborea* collected from 0 and 10 m (Fig. 4). Photosynthesis versus irradiance responses were modeled with the Michaelis–Menten formulation, i.e. a rectangular hyperbola (Miller 2002). Maximum photosynthetic rates based on both O_2 evolution and carbon uptake were 22 and 33% less, respectively, in 10 m kelp compared to surface-dwelling kelp (Table 2). If differences in temperature and season are considered, P_{max} values for 0 m *L. hyperborea* calculated from micro-optode oxygen measurements (2.20 μmol O_2 m^{-2} s^{-1}; April 5°C) compare well with literature values from Clark-type O_2 electrode incubations. For example, at 12°C, P_{max} ranged from 2.4 to 2.9 μmol O_2 m^{-2} s^{-1} for *L. hyperborea* collected in May/June in Helgoland, Germany (Lüning 1979), and, at 13°C, P_{max} yielded 2.87 μmol O_2 m^{-2} s^{-1} in October for the same shallow-water kelp population at Finnøy, Norway (Miller 2002). Similarly, ^{14}C uptake rates produced 1.76 μmol C m^{-2} s^{-1} in the basal adult lamina (from 6 m depth) of *L. solidungula* from the Alaskan Beaufort Sea (Dunton & Jodwalis 1988), which is comparable to 1.92 (0 m) and 1.32 (10 m) μmol C m^{-2} s^{-1} determined for *L. hyperborea* using the ^{13}C method.

In all light treatments, carbon assimilation rates were significantly lower than gross O_2 evolution rates (paired t-test; $p < 0.001$); therefore, photosynthetic quotients (PQ) were >1. PQ is defined as the mole ratio of rates of O_2 evolution and C uptake. In *Laminaria hyperborea*, PQ ranged from 1.14 for 0 m kelp to 1.30 for 10 m kelp, though these values were not significantly different (Table 2). Both values are within the range of other PQ estimates (0.67 to 1.57) in the Laminariales (Axelsson 1988, Hatcher et al. 1997).

Table 2. *Laminaria hyperborea*. Gross maximum photosynthesis (gP_{max}), oxygen respiration (R_d) and photosynthetic quotient (PQ) from kelp collected at 0 and 10 m depth. Statistical p-values result from independent t-tests for means between collection depths. P_{max} values are means (±SE) of n = 5 independent Michaelis-Menten curve fits

Measure	0 m	10 m	p
gP_{max} oxygen (μmol O_2 m^{-2} s^{-1})	2.20 ± 0.31	1.72 ± 0.35	0.048
gP_{max} carbon (μmol O_2 m^{-2} s^{-1})	1.92 ± 0.20	1.32 ± 0.21	0.002
R_d oxygen (μmol O_2 m^{-2} s^{-1})	−0.83 ± 0.16	−0.61 ± 0.09	0.233
PQ	−1.14 ± 0.07	1.30 ± 0.15	0.068

Table 3. Analytical experimental error associated with ^{13}C uptake measurements. Sample means represent replicate measurements of the same variable for a photosynthetic rate calculation. Data were taken from the calculation of photosynthesis for a single, randomly chosen, light-saturating treatment with a 10 m *Laminaria hyperborea* tissue disc (actual p = 1.11 µmol C m^{-2} s^{-1}). In the sensitivity test, P' was calculated assuming that the measurement erred positively by the coefficient of variation (CV), and percent deviation of P' from P is reported for comparison. ND: no data

Variable	Description	Mean ± SD (n)	CV (%)	*P* sensitivity to mean + CV P' (µmol C m^{-2} s^{-1})	Deviation (%)
a_{12b} (%)	at.% ^{13}C $NaH^{12}CO_3$ stock	1.1047 ± 0.0001 (3)	0.01	1.11	0.01
a_{sd} (%)	at.% ^{13}C diluted seawater	2.3271 ± 0.0114 (9)	0.49	1.10	–0.93
DIC_{se} (mM)	ΣCO_2 enriched seawater	2.51 ± 0.03 (10)	1.02	1.13	1.00
a_{tf} (%)	at.% ^{13}C final algal tissue	1.4774 ± 0.0029 (4)	0.19	1.12	0.75
a_{tn} (%)	at.% ^{13}C natural algal tissue	1.0896 ± 0.0009 (4)	0.08	1.11	–0.24
W (%)	Thallus weight percent C	28.6 ± 0.5 (4)	1.76	1.13	1.76
D (mg)	Thallus dry weight	12.7 ± 0.1 (4)	0.79	1.12	0.79
α	Fractionation factor	1.020 ± 0.001 (4)	0.09	1.12	0.09
B (m^2)	Biomass (area)	2.54×10^{-4}	ND	ND	ND

Macroalgal dry weight (*D*) and weight percent carbon (*W*) were significantly different in 0 and 10 m collected kelp. Shallow-water kelp have 32.7% greater dry weight per unit area (mean 0 m = 16.0 mg cm^{-2}, 10 m = 10.7 mg cm^{-2}; *t*-test: $p < 0.001$) and 9.7% more carbon by weight (mean 0 m = 34.3%, 10 m = 31.0%; *t*-test: $p < 0.001$) than 10 m collected kelp. As carbon weight percent is proportional to the peak area of the EA-IRMS signal, both *D* and *W* were determined individually for each experimental kelp disc without subsampling. Thus, disc-specific values of *D* and *W* reduced variation in photosynthetic rate compared to rates calculated using population means of the 2 measurements.

Variations in photosynthesis calculations originate primarily from inter-thallus differences rather than analytical error of the ^{13}C method. Intra-sample variations in *P* were determined for a single ^{13}C light treatment (Table 3). If variation is defined as the CV and if *P* was calculated assuming each variable equaled its mean plus CV, then the recalculated P' values changed by <2% for all analytical variables. Even if all measurements coincidently varied by their respective CV — in the direction that would change *P* positively — P' increased a maximum of only 5.7%. Measurements of a_{tf}, a_{tn} and a_{sd} are the most critical to the final *P* value, but intra-sample measurement variations in each of these 3 variables were <0.5%. In contrast, replicate kelp thalli in identical light treatments have 16.8% CV averaged over all light treatments (^{13}C method), which is less than the 22.6% inter-thallus variation measured with O_2 micro-optodes (*t*-test: p = 0.023). Further, this approximate 20% variation in photosynthesis is consistent with the variation found in basal lamina dry weights (25.1% in 0 m kelp and 23.3% in 10 m kelp). Inter-thallus P_{max} variation is reduced somewhat if P_{max} is calculated from independent Michaelis-Menten curve fits (O_2: 0 m 14.0%, 10 m 20.3%; C: 0 m 10.5%, 10 m 16.3%; Table 2). Regardless, analytical error of the ^{13}C method remains small compared to natural heterogeneity of the thallus.

DISCUSSION

Micro-optode O_2 measurement and the ^{13}C labeling procedure produced photosynthetic rates in *Laminaria hyperborea* that agree with published values and were mutually consistent, assuming a reasonable PQ. Intrinsic physiological heterogeneity explained most of the procedural variation in photosynthetic rates, and photosynthetic variation from 17 to 23% was comparable to the 23 to 25% variation in simple measurements of lamina dry weight.

Oxygen micro-optode based photosynthetic procedures, methodological considerations, and rate calculations are similar to established O_2 electrode protocols (Littler & Arnold 1985). Micro-optodes offer compact oxygen systems that provide rapid O_2 assessment without concerns of background electrical interference or oxygen consumption by the probe. Optode technology is improving with current availability of oxygen-sensitive foils, which, once embedded with a incubation chamber, offer a non-invasive measurement of O_2. Regardless, oxygen measurements remain critical to many macrophyte metabolic studies.

Photosynthetic carbon assimilation based on ^{13}C uptake is an alternative to ^{14}C primary production experiments in macroalgae. A significant advantage of using a ^{13}C label is the avoidance of hazards and restrictions associated with radioactive ^{13}C isotopes. Stable ^{13}C is readily transported across international borders and does not produce radioactive waste, both

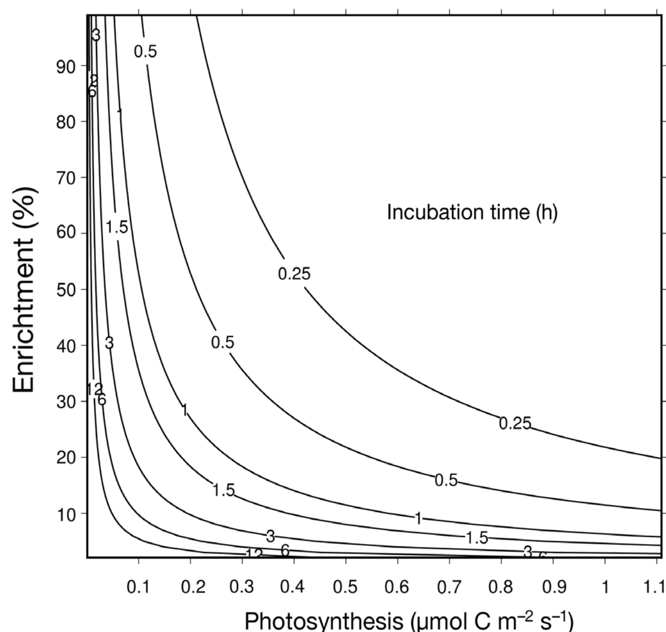

Fig. 5. *Laminaria hyperborea*. Sensitivity of the ^{13}C method using a 10 m kelp sample. Minimum incubation time needed to resolve photosynthetic rates in various ^{13}C-enriched media is shown. Rates were considered resolved when a_{tf} was significantly different than a_{tn} (Eq. 7), assuming a 1-tailed *t*-test with mean, variances, and experimental data in Table 3

significant impediments when applying ^{13}C procedures. Further, the non-radioactive ^{13}C method simplifies experimental protocols in the laboratory and in the field. After incubation, tissue samples are simply dried, weighed, ground to powder, and analyzed on an EA-IRMS. In addition, since $\delta^{13}C$ and $\delta^{15}N$ can be determined concurrently on an EA-IRMS, dual labeling experiments with ^{13}C and ^{15}N are possible and would address both carbon and nitrogen metabolism (Slawyk et al. 1977, 1988). On a larger scale, combined ^{13}C and ^{15}N enrichment experiments can be used to trace macrophyte/microphyte C and N fluxes through grazer or DOM systems (Hama et al. 1993, Herman et al. 2000).

Cost and sensitivity are 2 disadvantages of the ^{13}C technique. At an estimated US \$20 sample^{-1}, cost may become prohibitive with complex experimental designs that require high sample replication. An advantage of ^{14}C is its great sensitivity in resolving metabolic rates at short time scales and with limited alteration of seawater chemistry. If method sensitivity is defined as experimental time needed to measure a given rate at a given seawater at.% ^{13}C enrichment, the ^{13}C method is most feasible during several hour incubations and with higher photosynthetic rates (Fig. 5). These conditions require less ^{13}C enrichment and, thus, less pre-treatment of the incubation medium. In our 1.5 h incubations, we expected to be able to measure rates <10% P_{max} but needed media at.% ^{13}C to be >50%. It then became necessary to allow media to partially re-equilibrate with atmospheric DIC concentrations, and we were required to directly measure initial seawater $\delta^{13}C$ and DIC concentration for each trial.

ΣCO_2 enrichment

Unlike ^{14}C tracer methods, addition of ^{13}C label to incubation media significantly increases seawater ΣCO_2. A substantial enrichment in a_{se} is necessary to generate measurable increases in the isotopic composition of tissue organic carbon at the end of the incubation, and the relative ^{13}C differences between light treatments increase with more at.% ^{13}C added to the incubation medium. Procedurally, the actual increase in inorganic ^{13}C, and therefore ΣCO_2, depends on incubation time, i.e. less initial enrichment is necessary for longer time trials (Fig. 5).

Hama et al. (1983, 1993) argue that since phytoplankton are generally not carbon limited at seawater ΣCO_2 concentrations, the increase in ΣCO_2 in ^{13}C procedures has no significant effect on the determination of photosynthetic rates. As evidence, Hama et al. (1983) compared photosynthetic rates of phytoplankton using various increased inorganic carbon concentrations, up to 17.7% of seawater ΣCO_2, and found that ^{13}C-measured photosynthetic rates changed little with more available ΣCO_2. Even so, the assumption of no carbon limitation in macroalgae may not apply in all situations. Carbon limitation may arise in 2 experimental (and natural) conditions: (1) formation of a diffusive boundary layer around the thallus (Wheeler 1980, Hurd 2000) and (2) exhaustion of inorganic carbon supply, as in tide pools (Maberly 1990). In the former case, boundary layer thickness is minimized with sufficient water motion in incubation containers (Littler 1979). In the latter, if algae are able to utilize HCO_3^-, inorganic carbon will likely not become limiting in enriched ^{13}C experiments. On the other hand, artificially high ΣCO_2 in ^{13}C experiments may dampen naturally significant photorespiration (Holbrook et al. 1988).

To ameliorate confounding problems with high inorganic carbon concentrations, we allowed our incubation medium to partially re-equilibrate with the atmosphere. Incubation ΣCO_2 was decreased further when O_2 was purged with N_2 prior to incubations. Consequently, ΣCO_2 levels in our incubation media were <17% over natural concentrations, a ΣCO_2 enrichment which likely did not accelerate production rates (Mateo et al. 2001). Both manipulations required that

a_{se} and DIC_{se} were determined analytically prior to the start of each incubation rather than being calculated directly from the quantity of ^{13}C initially added to the medium (Table 1). An alternative approach that would ensure better control over both the degree of ^{13}C enrichment and inorganic carbon content would be to replace natural ΣCO_2 with amended ΣCO_2. This can be achieved by acid-stripping seawater (see 'Materials and methods; Incubation medium carbon isotope measurement'), re-establishing pH, and then adding appropriate qualities of ^{12}C and ^{13}C bicarbonate to reach a target ΣCO_2 concentration and ^{13}C enrichment.

^{13}C uptake measures gross not net photosynthesis

The general procedure and underlying biochemistry of carbon uptake experiments are similar for both ^{13}C and ^{14}C techniques, and both require the same methodological considerations (Peterson 1980, Arnold & Littler 1985). Of chief concern is whether carbon isotope procedures measure gross or net photosynthesis, or something in between. The discrepancy arises primarily because, unlike O_2, labeled organic carbon is not immediately available as a substrate for respiration (Hobson et al. 1976, Dring & Jewson 1982). There is a time lag, on the scale of hours (Dring & Jewson 1982), before labeled photosynthetic products become accessible to respiratory cycles. As a result, ^{14}C labeling measures gross photosynthesis, at least in the short term. In macroalgae, the argument of gross photosynthesis is reinforced as respiratory carbon pools are large and have long turnover times compared to phytoplankton (Søndergaard 1988). In an experiment with 12 species of macroalgae, Søndergaard (1988) concluded that ^{14}C incubations measured gross photosynthesis for up to 33 h. The same arguments should apply to stable isotope tracer experiments. Short-term ^{13}C experiments will be a measure of gross photosynthesis, and therefore caution should be used when extrapolating gross carbon uptake rates to long-term or community-level productivity.

DOM exudation

Arnold & Littler (1985) stress the importance of quantifying the amount of dissolved organic matter (DOM) released in ^{14}C experiments. For example, fucoid exudation, determined with minimal disruptive techniques, averaged 2 to 5% of daily production (Carlson & Carlson 1984), though wounding may increase DOM and mucilage release. Release of DOM likely has little effect on the final photosynthetic rate for the same reasons that ^{13}C procedures measure gross photosynthesis: namely, it is speculated that labeled photosynthetic products are not available for exudation on short time scales. Nevertheless, quantifying DOM exudation would strengthen the overall ^{13}C procedure.

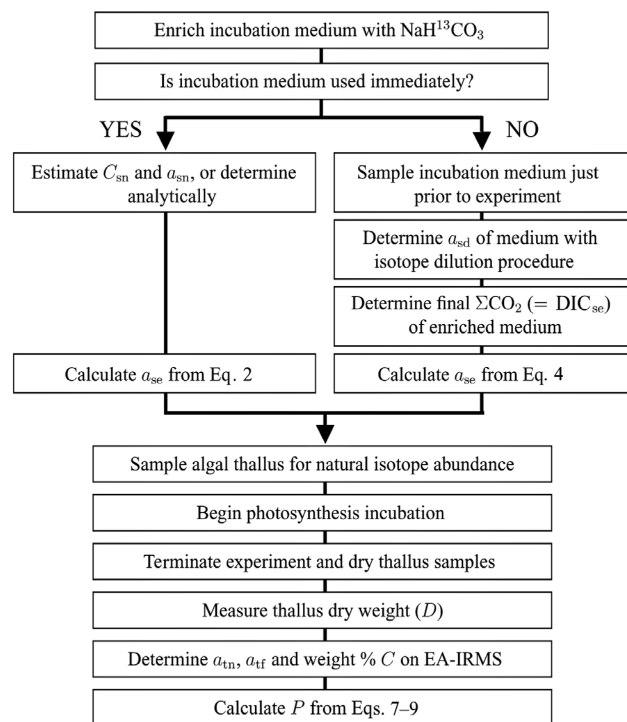

Fig. 6. ^{13}C method diagram for photosynthetic rate determination

CONCLUSIONS

Marine ecologists are concerned with how carbon flows through primary producers to grazers and other consumers found in an ecosystem (Trancoso et al. 2005). The first step of the process is to quantify how much carbon is assimilated into plant/algal organic tissue through photosynthesis. *In situ* procedures are generally preferred, since they reproduce natural environmental and physiological conditions to a better extent than laboratory- or culture-based methods. We applied 2 techniques for measuring photosynthesis in macroalgae, both of which are suited for field studies (Mousseau et al. 1995, Glud et al. 1999). Without the hazards and restriction of ^{14}C tracer experiments, the ^{13}C technique should encourage direct measurement of carbon assimilation in macroalgae. The general ^{13}C procedure is straightforward and should readily be adapted to other experimental configurations (Fig. 6). Where O_2 photosynthetic mea-

surements are desirable and where studies require that both carbon and oxygen metabolic rates are determined concurrently, the micro-optode oxygen instrument provides a compact system for rapid quantification of dissolved oxygen.

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