European Journal of Phycology

ISSN: 0967-0262 (Print) 1469-4433 (Online) Journal homepage: https://www.tandfonline.com/loi/tejp20

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To cite this article: John W. Runcie , Carlos F.D. Gurgel & Karla J. Mcdermid (2008) *In situ* photosynthetic rates of tropical marine macroalgae at their lower depth limit, European Journal of Phycology, 43:4, 377-388, DOI: 10.1080/09670260801979303

To link to this article: https://doi.org/10.1080/09670260801979303

Published online: 27 Nov 2008.

Eur. J. Phycol., (2008), **43(4)**: 377–388

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In situ photosynthetic rates of tropical marine macroalgae at their lower depth limit

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(Received 30 September 2007; accepted 11 February 2008)

Most photophysiological studies of marine macroalgae have focussed on algae in waters shallower than 30 m. However some species are abundant at depths in excess of 100 m with irradiances less than 1 μmol photons $m^{-2} s^{-1}$. We examined, for the first time, the *in situ* efficiency of photochemical energy conversion of a variety of epilithic macroalgal species at depths from 86 to 201 m using a piloted submersible and multiple-turnover modulated chlorophyll fluorescence measurements based on the PAM technique. The irradiance at which electron transport rate reached a maximum (Ek) for green algae declined from 50 μmol photons $m^{-2} s^{-1}$ (at ~90 m) to less than 10 μmol photons $m^{-2} s^{-1}$ at their lower depth limit of 140 m; photochemical quenching in response to light exposure declined markedly at depths below 100 m, while non-photochemical quenching remained low at all depths, indicating minimal photoprotective capacity in these algae. Values of Ek for encrusting Corallinales at 201 m were ~4 μmol photons $m^{-2} s^{-1}$, which exceeded by 400 times the maximum ambient irradiance at that depth. In the short term, the deep-water red algae examined (in particular the encrusting species) were able to tolerate and take advantage of irradiances orders of magnitude greater than the estimated noonday surface irradiance. Non-photochemical quenching of the red algae also increased with depth, indicating these algae retain their capacity for coping with high light even when in very deep waters. Carbon stable isotope data of deep algae confirmed the diffusion of inorganic carbon with its minimal energy requirement is probably the primary means of inorganic carbon uptake. The observed lower depth limits of selected macroalgae at Penguin Bank are shallower than depth limits for comparable species reported in the literature. Occasional smothering of algae by sediment, observed at Penguin Bank, would reduce the annual photon dose, thereby reducing the depth limit.

Abbreviations

E:	irradiance in μmol photons $m^{-2} s^{-1}$
Ek:	the irradiance where electron transport rate reaches a theoretical maximum
rETR:	relative electron transport rate
rETRmax:	relative maximum electron transport rate
Fm′:	maximal fluorescence during saturating pulse of light
Ft:	minimal fluorescence immediately before saturating pulse
Kd:	attenuation coefficient of seawater
NPQ:	non photochemical quenching (Stern-Volmer model)
PAR:	photosynthetically active radiation
qN:	non-photochemical quenching
qP:	photochemical quenching
RLC:	rapid light curve
ΔF/Fm′:	the effective quantum yield of chlorophyll fluorescence as measured by (Fm′ – Ft)/Fm′.

Key words: chlorophyll fluorescence; deepwater; electron transport rate; epilithic macroalgae; Corallinales; Hawai'i; *in situ*; effective quantum yield; *Ulva*;

Introduction

The discovery of abundant autotrophic macroalgae living at 268 m on an uncharted seamount in the Bahamas, where irradiance was 0.0005% that of surface irradiance (Littler *et al.*, 1985, 1986) prompted revision of the widely held concept that the minimum irradiance required for photosynthesis was 1% of surface irradiance (Ryther, 1956). Subsequent studies using dredging, piloted submersibles and remotely operated vehicles have focused on examining the role of deep-water

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ISSN 0967-0262 print/ISSN 1469-4433 online/08/040377–388 © 2008 British Phycological Society
DOI: 10.1080/09670260801979303

macroalgae in deep-sea food webs, primary productivity, reef building, sediment production (Hillis-Colinvaux, 1986; Littler *et al.*, 1991), and the discovery of new species (Joly & Oliveira, 1967; Aponte & Ballantine, 2001 and references therein). Deep-water macroalgae are now recognised as an important component of many coastal ecosystems, offering substratum for reef building, habitat for motile species, and food for herbivorous fish and benthic bacteria.

Fewer studies have examined the physiology of deep-water macroalgae. To our knowledge, physiological measurements made on algae at depths greater than 50 m are restricted to *in vivo* observations on material collected and brought to the surface (e.g. Littler *et al.*, 1985; Titlyanov *et al.*, 1992; Costa-Braga & Yoneshigue-Valentin, 1994; Rodrigues *et al.*, 2000, 2002), even though a large proportion of the nearshore benthic environment is at depths from 50 to 200 m. *In situ* and *in vivo* experiments have focussed on estimating photosynthetic capacity and the proportion of oxygenic photosynthesis to carboxygenic respiration (e.g. Titlyanov *et al.*, 1992). However, these experiments do not necessarily reflect the physiological poise of macroalgae in their natural environment. Requirements for accurately simulating the deep-water environment (low irradiance, lower temperature, increased gas solubility), especially during transfer to the surface, can be difficult to meet. Ideally, physiological studies of deep-water algae would be conducted *in situ*, and this has been made possible with recent advances in technology.

Strategies employed by deep-water macroalgae that enable them to survive low-light environments include larger photosynthetic unit sizes, limited PSII repair cycles, smaller pools of xanthophyll carotenoids, lower photosynthetic capacity, lower pigment content, thinner thalli (i.e. more photosynthetic components per unit mass), and apparent compensation between pigment content and photosynthetic capacity. Raven *et al.* (2000) reviewed in detail the physiological strategies employed by low-light living phototrophs. Low-light macroalgae are more prone to photodamage as they are less able to dissipate excess light energy via photochemistry due in part to a limited quantity of carbon fixation cycle enzymes, e.g. RuBisCO. Deep-water algae do not necessarily have more chlorophyll than shallower algae, rather they increase the efficiency of light harvesting when optimized for low-light intensities (Rodrigues *et al.*, 2000).

In situ experiments on macroalgae generally use SCUBA at depths to 30 m, although the relatively recent use of open- and closed-circuit, mixed-gas diving has enabled research at depths exceeding 50 m. To date, no studies have reported the *in situ* examination of physiological processes of benthic algae at depths beyond conventional SCUBA. In this study we conducted a series of submersible dives in the main Hawaiian Islands where we examined macroalgae from depths of 70 m to 201 m. We used a custom-built fluorometer to examine macroalgal photosynthetic efficiency ($\Delta F/Fm'$, the effective quantum yield of chlorophyll fluorescence). In addition we measured tolerance to irradiance by exposing the algae to artificial actinic light in the form of rapid light curves (RLCs; White & Critchley, 1999). *In situ* variable fluorescence measurements were complemented with *in situ* irradiance (PAR) measurements and twice daily Kd estimates during descent and ascent of the submersible. A time-series of $\Delta F/Fm'$ and associated quenching parameters were obtained by deploying a stand-alone fluorometer overnight on a single algal sample.

Methods

Sample collection

In situ variable fluorescence measurements and macroalgal collections were made using the Pisces V piloted submersible (Hawai'i Undersea Research Laboratory, University of Hawai'i) between 16–22 September 2004 and 29 September–1 October 2006 at depths ranging from 74 to 204 m. The study was conducted on the north-facing slope of a submerged ridge known as the 'Third Finger' at 20°56′W; 157°32′N, situated on the Penguin Bank, a submerged reef to the west to the island of Moloka'i in the Hawaiian archipelago. At each sampling site and, where possible, thalli from more than one individual alga were collected using the submersible manipulator and placed in numbered plastic jars within a light-proof box. At several locations only one individual alga could be sampled. Thalli were subsampled for elemental analysis, pigment analysis and taxonomic identification. Material for elemental and stable isotope analysis (C, N, $\delta^{13}C$, $\delta^{15}N$) were rinsed in deionized water and frozen at −20°C. Samples collected for pigment analysis were inadvertently degraded and could not be used. Remaining material was identified, or pressed or preserved in formaldehyde or silica gel for archival and DNA phylogenetic analyses. Specimens were identified to the lowest taxonomic level possible based on examination of morphological and anatomical characteristics using taxonomic references (Hillis-Colinvaux, 1980; Abbott, 1999; Abbott & Huisman, 2004).

Seaweeds collected included *Caulerpa mexicana* Sonder ex Kützing, *Cladophora* sp., *Codium*

mamillosum Harvey, encrusting members of the Corallinales, *Cryptonemia* sp., *Distromium flabellatum* Womersley, *Halimeda distorta* (Yamada) Hillis-Colinvaux, *Kallymenia* sp., *Microdictyon umbilicatum* (Velley) Zanardini, *Osmundaria* sp., *Peyssonnelia inamoena* Pilger, *Spatoglossum macrodontum* J. Agardh and *Ulva expansa* (Setchell) Setchell & N.L. Gardner. New records will be described in a separate paper. Voucher specimens were selected and deposited in the Bishop Museum Herbarium in Honolulu (BISH).

Irradiance measurements

Irradiance was measured with a LiCor underwater cosine sensor (Li192SA) attached to the submersible. Readings were taken at intervals during descent and ascent (and throughout each dive), and the attenuation coefficient Kd was calculated from these data according to Kirk (1994). The annual and daily photon dose at each depth was then calculated assuming the annual photon dose at the earth's surface at 20°57′ latitude is 16,449 mol photons m^{-2} (Irradiance Simulator V1.01, Ed Drew ©2003). This model calculates solar radiation reaching the earth's surface and is based on the solar constant, declination of the sun, the zenith angle and time and latitude. The model assumes an air mass of 1 (sun is directly overhead), and that 9% of the solar beam irradiance is back-scattered from the atmosphere, generating the diffuse irradiance. Calculations were made assuming clear skies.

Stable isotopes of C and N

While in most cases at least two individual thalli were analysed for C, N and their stable isotopes, at some locations only a single algal thallus was available for analysis. Values of C, N, $\delta^{13}C$ and $\delta^{15}N$ were (generally) calculated as the mean of two or more individual thalli. Where only one thallus could be collected, data are reported to indicate this. Algal samples with external carbonaceous structures were treated with dilute HCl to remove inorganic carbonate. Carbon and nitrogen content (percent) and stable isotope composition ($^{15}N:{}^{14}N$ and $^{13}C:{}^{12}C$) were determined using a Carla Erba NC2500 Elemental Analyzer and a Finnigan MAT ConFloII system. $\delta^{13}C$ and $\delta^{15}N$ were calculated with Pee Dee Belemnite and atmospheric nitrogen as standards. The δ notation refers to the proportion of stable isotopes (of C and N) in the sample relative to the proportion of isotopes in the standard, and indicates the extent of fractionation of these elements that is represented by the alga relative to the standards:

$$\delta^{13}C = \left(\left[{}^{13}C/{}^{12}C\right]_{sample}\right)/\left(\left[{}^{13}C/{}^{12}C\right]_{PDB}\right)^{-1} \quad (1)$$

where sample refers to the alga examined, and PDB refers to the PeeDee Belemnite standard. $\delta^{15}N$ are calculated by substituting $^{15}N/^{14}N$ values of sample and atmospheric N as the standard.

Δ was calculated according to Mook *et al.* (1974), Maberly *et al.* (1992) and Raven *et al.* (1995):

$$\Delta = \left(\delta^{13}C_{CO2} - \delta^{13}C_{alga}\right)/\left(\delta^{13}C_{alga} + 1000\right) \quad (2)$$

where Δ represents the $\delta^{13}C$ value of the algal sample ($\delta^{13}C_{alga}$) relative to the $\delta^{13}C$ of the source of inorganic carbon used in photosynthesis (i.e. $\delta^{13}C_{CO2}$, the dissolved CO_2). While this relation does not distinguish between CO_2 uptake via diffusive or active pathways, it can provide an estimate of the extent to which the inorganic C has been fractionated during photosynthesis, thereby indicating which photosynthetic pathways have been active. Seawater physical parameters used to estimate seawater $\delta^{13}C_{(CO2)}$ (salinity, 35.23 psu; inorganic phosphate, 0.084 μmol kg^{-1}SW; inorganic silicate, 1.61 μmol kg^{-1}SW; temperature, 22.6°C; pressure, 100 dbar; DIC, 2006.6 μmol kg^{-1}SW; alkalinity, 2,323 μmol kg^{-1}SW) were derived from comparable oceanic data (Station ALOHA, Aug–Sep 2004, 98–102 m depth, http://www.soest.Hawai'i.edu/HOT_WOCE/index.html). These mean values corresponded well with measurements made at the study site during our visits in September 2004 and 2006. As most algae were collected from depths between 76 and 140 m, calculations were made assuming a common depth of 100 m.

In situ *variable fluorescence*

A custom fluorometer comprising a sensor unit linked via cable to a data-logger/controller was used in either stand-alone mode or the sensor was connected via cable to the data-logger within the command sphere of the submersible. Ready access via laptop to the data-logger enabled adjustment of fluorescence intensity from within the submersible to ensure adequate signal strength. The fluorometer sensor incorporated a blue (470 nm) LED to provide modulated excitation light, a white 1-W LED for actinic and saturating flashes (Lumiled, Luxeon), and a red longpass filter (Schott RG695) that ensured only red light (fluorescence) was received by the photodiode. All LEDs and the photodiode were directed through a transparent tube at the algal sample to be examined. The distance from the photodiode to sample was approximately 10 mm. The quantum yield of chlorophyll fluorescence during daytime ($\Delta F/Fm'$) was determined using the multiple-turnover

saturation–pulse technique (commonly known as the PAM technique) as described by Schreiber (2004), where the saturating pulse was 0.8 s in duration. Initial measurements of $\Delta F/Fm'$ were followed by a standard light treatment (rapid light curve [RLC]; White & Critchley, 1999; Ralph & Gademann, 2005), where the sample was subjected to eight 10 s intervals of actinic irradiance of increasing intensity with a maximum actinic intensity of 38 $\mu mol\, m^{-2}\, s^{-1}$.

The most practical method of positioning the fluorometer sensor using the manipulator arm of the submersible was simply to place the sensor over an algal sample. Consequently, the distance between sample and actinic LED varied between RLCs, resulting in variation in actinic irradiance of the individual RLC steps from sample to sample. As we had no way of accurately characterizing this variation, RLCs were calculated assuming a fixed distance between sample and actinic source and the average of at least three RLCs was obtained for each species at a location. Each of these three RLCs was performed on a new and separate alga that had not been previously measured.

Diel stand-alone measurements of variable fluorescence of *U. expansa* were effected by deploying a sensor connected to a custom submersible data-logger; the sensor was installed in a clear sample holder that facilitated operation by the submersible manipulator and allowed an algal sample to be exposed to the water column and ambient irradiance during the period of measurements. Signal strength was monitored during setup and adjustments to the sample position made using the manipulator as required. The device was programmed to conduct RLCs every 90 min, and photochemical (qP) and non-photochemical (qN, NPQ) quenching calculated from the last $\Delta F/Fm'$ values of the RLC described the response of the photosystem to the RLC light treatment. While it was our intention to measure multiple samples with multiple fluorometers, we were only able to measure one algal thallus at 107 m. Estimates of error for these values were obtained from similar measurements on groups of *U. expansa* (see below).

Data analysis

Relative ETR (rETR) was calculated simply as the product of PAR and $\Delta F/Fm'$. RLCs were described according to Webb *et al.* (1974). The curve-fitting procedure used non-linear least-squares iterative techniques with Sigmaplot software (Jandel Scientific Inc. San Rafael, CA). Estimates of maximum rETR (rETRmax), the theoretical minimum saturating irradiance (Ek), and the slope of the ETR-E curve (alpha) were derived from these model fits. Approximations of qP, qN and NPQ at the end of each RLC were calculated according to Schreiber (2004). As we did not have direct measurements of Fm, Fo or Fo′ (Van Kooten & Snel, 1990), we assumed that values of qP and qN for the initial $\Delta F/Fm'$ measurement of each RLC were one and zero respectively.

Estimates of error for photosynthetic parameters of the single *Ulva expansa* thallus were obtained by determining the mean of the standard deviations of four groups of RLCs performed on *U. expansa* at different sites as a percentage of the mean parameter value. The (assumed) standard deviation associated with $\Delta F/Fm'$ of the deployed alga was then calculated using this percentage.

Results

Distribution of species

Foliose algae were found as deep as 140 m. In deeper water only encrusting coralline species were observed to 200 m depth. Dives were limited to this depth in order to prevent damage to the fluorometer. Table 1 lists the lowest depths where each species was observed. Note that the lowest depth limits recorded here are only derived from a limited set of observations – an extensive survey of algal distribution was beyond the scope of this study.

Irradiance and temperature

Measurements of irradiance attenuation with increasing depth at Penguin Bank over one week provided a mean Kd value of 0.046 (0.0395 to 0.0476), typical of very clear oceanic water (Jerlov oceanic water type 1). Surface waters were generally 27.5°C while deep water was cooler (e.g. 24.3°C at 100 m, 18.5°C at 200 m). The annual and daily photon dose for species collected both in this and other studies are presented in Table 1.

Elemental and stable isotope ratios

Of all the species examined, only *M. umbilicatum* demonstrated a clear decline in C : N and $\delta^{15}N$ with increasing depth; $\delta^{13}C$ remained relatively constant across depths (Table 2). While the encrusting coralline algae demonstrated $\delta^{13}C$ values as high as +1.22‰, *Cryptonemia* sp. had the most negative value of –35.28. Most algae had values between –5‰ and –20‰. $\delta^{15}N$ ranged from 1.09 (*H. distorta*) to 4.95 (encrusting coralline alga at 201 m), the average value was 3.03. C : N of calcifying organisms such as *H. distorta* and the encrusting

Table 1. Annual and daily photon dose at apparent (this study) and cited maximum depths of deep-water macroalgae. Annual and (mean) daily photon dose at depth for cited species (Littler *et al*., 1986; Markager & Sand-Jensen, 1992; Middleboe & Markager, 1997; Aponte & Ballantine, 2001) were calculated according to estimated photon flux at the earth's surface at the appropriate latitude. Morphological groups are defined by Markager & Sand-Jensen (1992), irradiance in the cave referred to in this study was undefined.

Species	Latitude	Depth (m)	Annual photon dose (surface irradiance) (mols m^{-2})	% Surface irradiance	Annual photon dose (depth) (mols m^{-2})	Daily photon dose (depth) (mols m^{-2})
This study						
Caulerpa mexicana	20	83	16,449	2.2	361	0.99
Codium mamillosum	20	93	16,449	1.4	228	0.63
Cladophora sp.	20	94	16,449	1.3	218	0.59
Cryptonemia sp.	20	100	16,449	1.0	165	0.45
Spatoglossum macrodontum	20	107	16,449	0.73	120	0.33
Peyssonellia inamoena	20	115	16,449	0.50	83	0.23
Halimeda distorta	20	118	16,449	0.44	72	0.20
Ulva expansa	20	121	16,449	0.38	63	0.17
Distromium flabellatum	20	121	16,449	0.38	63	0.17
Osmundaria sp.	20	138	16,449	0.18	29	0.081
Microdictyon umbilicatum	20	140	16,449	0.16	26	0.072
crustose Corallinales	20	201	16,449	0.01	1.6	0.0045
Markager & Sand-Jensen, 1992						
Leathery						
Laminaria hyperborea	54	19	9,326	1	93	0.26
Laminaria hyperborea	54	8	9,326	0.7	65	0.18
Agarum cribrosum	43	40	11,969	0.44	53	0.14
Cystoseira sp	27	8.5	15,435	0.2	31	0.085
Cystoseira sp.	28	9.5	15,248	0.12	18	0.050
Laminaria solidungula	70	6.4	5,956	0.2	12	0.033
Laminaria solidungula	79	20	4,825	0.18	9	0.024
Foliose						
Monostroma sp.	21	118	16,444	0.22	36	0.099
Foliose macroalgae	11	140	17,612	0.18	32	0.027
Maripelta sp.	28	13	15,248	0.12	18	0.050
Ptilota serrata	43	50	11,969	0.11	13	0.036
Phycodrys rubens	43	50	11,969	0.11	13	0.036
Lobophora variegata	16	140	17,112	0.06	10	0.028
Johnson sea-linkia profunda	24	157	15,966	0.02	3	0.0087
Crustose						
Lithothamnion sp.	54	15	9,326	0.05	4.7	0.013
Leptophytum laeve	43	63	11,969	0.02	2.4	0.0066
Crustose red	21	182	16,444	0.008	1.3	0.0036
Corallines	11	228	17,612	0.004	0.7	0.0019
Crustose red	36	cave	13,587	0.003	0.41	0.00022
Crustose red	16	250	17,112	0.00023	0.04	0.00011
Aponte & Ballantine, 2001						
Halimeda incressata	23	36	16,002	13	2131	5.70
Rhipochephalus phoenix	23	36	16,002	13	2131	5.70
Udotea sp.	23	36	16,002	13	2131	5.70
Dictyota spp.	23	48	16,002	6.8	1088	2.98
Avrainvillea sp.	23	62	16,002	3.1	497	1.36
Microdictyon marinum	23	62	16,002	3.1	497	1.36
Halimeda discoidea	23	66	16,002	2.5	397	1.10
Sargassum sp.	23	66	16,002	2.5	397	1.10
Lobophora variegata	23	76	16,002	1.4	227	0.61
Halimeda copiosa	23	109	16,002	0.2	36	0.088
Verdigellas peltata	23	130	16,002	0.07	11	0.031
Corallinales	23	167	16,002	0.009	1.4	0.0039
Peyssonelia sp.	23	167	16,002	0.009	1.4	0.0039
Ostreobium sp.	23	200	16,002	0.001	0.22	0.00044
Littler *et al*., 1986						
Lobophora variegata	24	90	15,966	0.6	103	0.26
Halimeda copiosa	24	130	15,966	0.07	11	0.031
Peyssonnelia sp.	24	189	15,966	0.003	0.4	0.0013
crustose corallines	24	268	15,966	0.0005	0.005	0.0001
Middleboe & Markager, 1997						
Freshwater bryophyte	39	124	14,500	0.1	19	0.040

Table 2. Stable isotopes of C and N, and C/N ratios of macroalgae collected at a range of depths at Penguin Bank, Hawai'i in September 2004. δ^{13}C and δ^{15}N values are expressed relative to Pee Dee Belemnite and atmospheric nitrogen. Δ represents the extent of biological fractionation once the seawater C has entered the alga. Values represent the mean of two duplicate measurements derived from a single thallus ($n = 1$), or the mean of at least two measurements derived from at least two separate thalli ($n > 1$). nd, no data.

Species	ID#	Depth (m)	*n*	$^{13}\delta$C (‰)	$^{15}\delta$N (‰)	Δ (‰)	C : N
Chlorophyta							
Microdictyon umbilicatum	9144	91	2	−18.73	3.77	10	13.37
	9140	92	1	−18.90	3.79	10	13.93
	9139	94	1	−19.44	2.83	10	12.17
	9155	122	2	−18.91	2.20	10	11.13
	9022	140	2	−19.01	2.47	10	10.63
	9175	132	1	−19.32	2.02	10	11.10
Caulerpa mexicana	9072	83	2	−20.46	4.79	12	15.89
Cladophora sp.	9046	94	1	−17.25	3.75	8	9.79
Codium mamillosum	9052, 9047	92	2	−19.33	1.39	9,11	11.84
Halimeda distorta	9076	83	2	−2.52	1.11	−7	57.95
	9170	76	2	−7.37	2.80	−2	22.96
	9141	90	1	−1.02	2.19	−8	51.00
	9024	92	1	−5.97	2.29	−3	21.28
	9138	94	1	−7.79	2.35	−1	21.96
	9059	99	2	−0.64	2.11	−9	50.78
	9129	100	1	−6.10	1.09	−3	23.78
	9156	113	2	−2.61	1.51	−7	34.91
Ulva expansa	9132	88	1	−19.81	3.85	11	17.78
	9053	91	2	−17.42	3.71	8	12.58
	9048	93	2	−19.88	3.63	11	13.12
	9145	94	1	−19.42	3.75	10	11.84
	9013	95	1	−19.53	3.56	11	10.57
	9096, 9097, 9151	102	5	−18.40	3.59	10	8.77
Rhodophyta							
Osmundaria sp.	9174	132	2	−33.60	2.17	25	9.64
Peyssonnelia inamoena	9176	91	1	−14.48	3.14	5	15.38
	9150	102	2	−15.93	3.51	7	11.73
	9173	115	1	−9.77	2.96	1	18.62
	9134	120	2	−13.83	3.08	5	15.41
Cryptonemia sp.	9133	100	2	−35.28	3.63	27	8.12
Corallinales	9171	76	2	−3.86	2.33	−5	61.24
	nd	121	2	1.07	4.52	−10	215.48
	9127	137	2	−19.02	2.74	10	10.46
	9157	163	2	0.36	5.89	−10	75.11
	9158	201	1	1.21	4.95	−10	105.34
Phaeophyta							
Spatoglossum macrodontum	9142, 9002	86	2	−28.45	3.45	20	13.24
	9023, 9093	107	3	−23.44	3.03	15	11.675
	9137	131	1	−21.35	3.69	12	11.50
Distromium flabellatum	9095	121	1	−20.88	3.38	12	17.41

Corallinales was highly variable (despite acidification of samples prior to analysis in order to remove inorganic carbon). Only *Osmundaria* sp. and *Cryptonemia* sp. had values of Δ exceeding 20‰ (Table 2).

Variable fluorescence

With increasing depth, the maximum rate of relative electron transport (rETRmax) and the saturation irradiance (Ek) declined for all major groups of algae (Figs 1, 2). Values of Ek were consistently in excess of the maximum irradiance encountered at the depth where it was determined, for example while the maximum estimated ambient irradiance at 201 m was 0.01 μ mol photons $m^{-2} s^{-1}$, the Ek calculated from the rapid light curve treatment was 4.2 ± 2 μ mol photons $m^{-2} s^{-1}$. In contrast to the decline in rETRmax and Ek with depth, the photon conversion efficiency of the green algae (as measured with ΔF/Fm′ and alpha) did not vary significantly with increasing depth (from 88 to 137 m), with mean values of 0.693 and 0.775 respectively. The red and brown algae, and the crustose Corallinales maintained relatively constant values (ΔF/Fm′ 0.638, alpha 0.690; and ΔF/Fm′ 0.599, alpha 0.667 respectively).

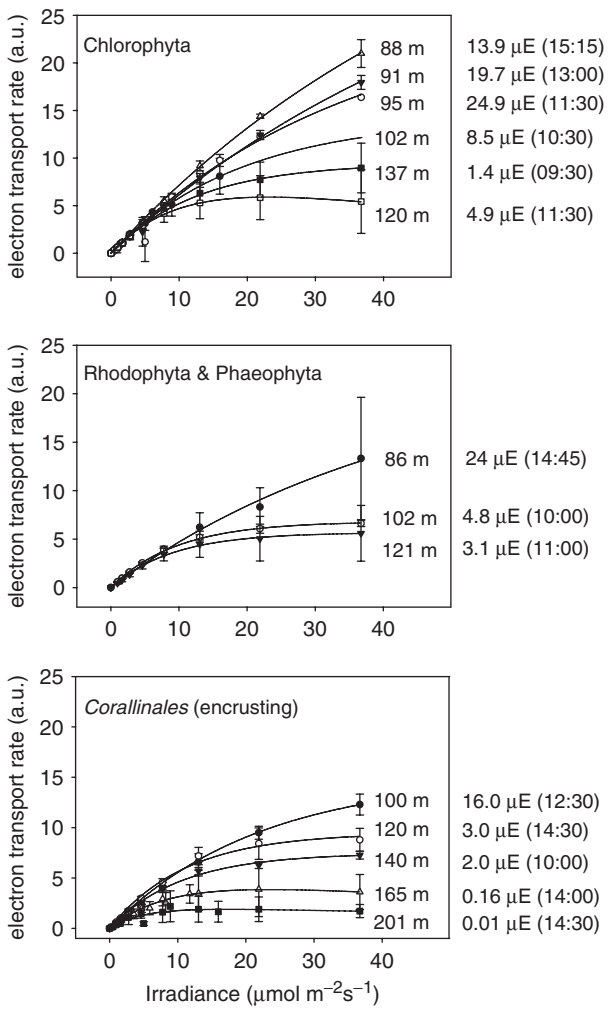

Fig. 1. *In situ* rapid light curves of macroalgae at Penguin Bank, Hawai'i at depths between 86 and 201 m. Chlorophyta (*Microdicyton umbilicatum, Ulva expansa*), Rhodophyta (*Cryptomenia* sp., *Peyssonnelia inamoena and Kallymenia* sp.) and unidentified crustose Corallinales were examined. Curves are means of at least three curves ± SD, where each curve was performed on a separate and individual alga. Depths are shown; irradiances and times in brackets represent the ambient irradiance and time when the RLC was performed.

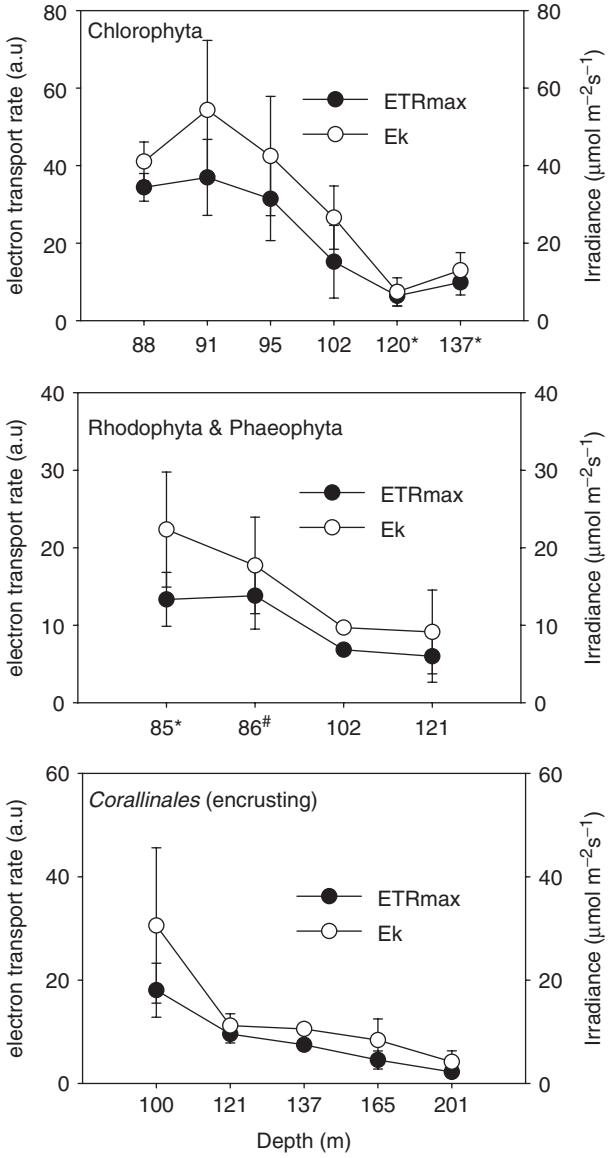

Fig. 2. Values of relETRmax and Ek derived from *in situ* RLCs conducted on macroalgae at depths from 85 to 201 m at Penguin Bank, Hawai'i. Note abscissa is not to scale. Data are means of at least three values ± SD, where each RLC was performed on a separate and individual alga.

At the end of each rapid light curve, both photochemical (qP) and non-photochemical quenching parameters (qN; VanKooten & Snel, 1990) were calculated, using values of Ft and Fm′ from the first measurement of effective quantum yield in the curve. qN of the red and brown algae increased with increasing depth, while qN of the green algae varied little between 88 and 138 m (Fig. 3). Conversely, qP of the red and brown algae declined with increasing depth (Fig. 3). A similar decline in qP of the green algae occurred markedly at depths below 100 m.

In situ *deployment*

Predicted ambient irradiance at the site of deployment (107 m) dropped from a maximum of ~40 µmol photons $m^{-2} s^{-1}$ at midday to darkness during the last two measurements after 18:00. Initial measurements were influenced by the submersible floodlights used to position samples – subsequent measurements are assumed to reflect better the physiological state of the sample *in situ*. While ΔF/Fm′ increased slightly after dark, alpha declined during the deployment (Fig. 4). Photochemical quenching declined between 30 and 40 percent after RLC-induced irradiances, and this decline was slightly greater after dark. Conversely, both non-photochemical quenching parameters increased as the day progressed with greatest values after dark. rETRmax and Ik were both greatest after dark. While the estimates of standard deviation of ΔF/Fm′ (Fig. 4) cannot be used to test for significance, these estimates

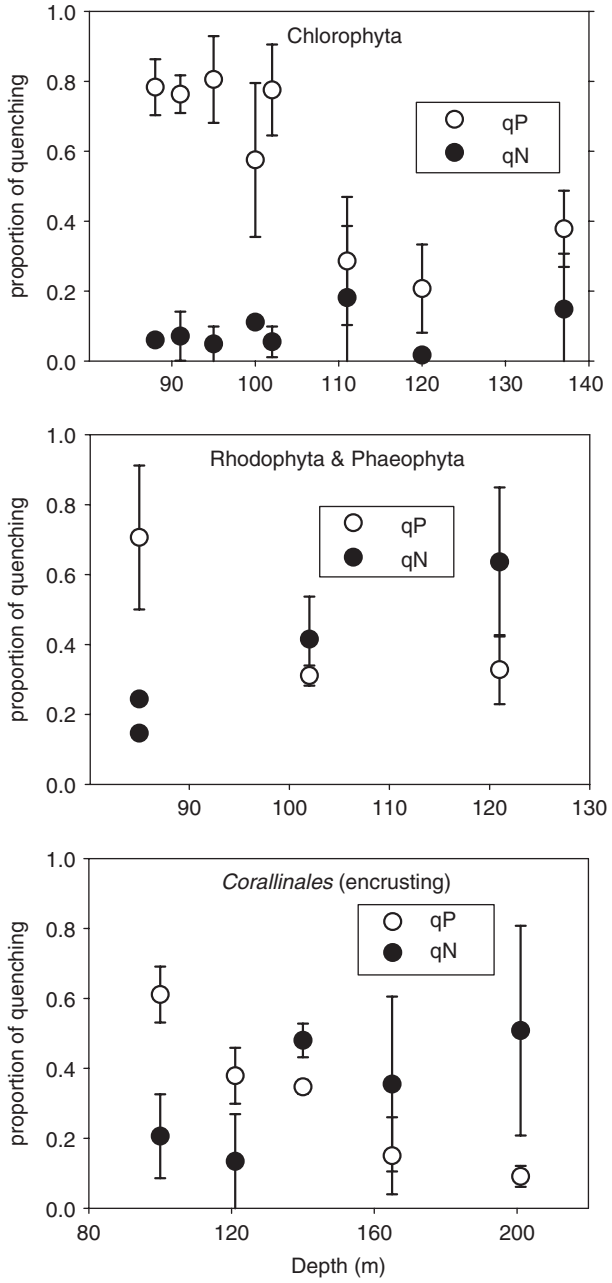

Fig. 3. Values of qP and qN derived from the last pulse of *in situ* RLCs conducted on macroalgae at depths from 85 to 201 m at Penguin Bank, Hawai'i. Note abscissa is not to scale. Data are means of at least three values ± SD, where each RLC was performed on a separate and individual alga (except where two data points and no error bar are shown).

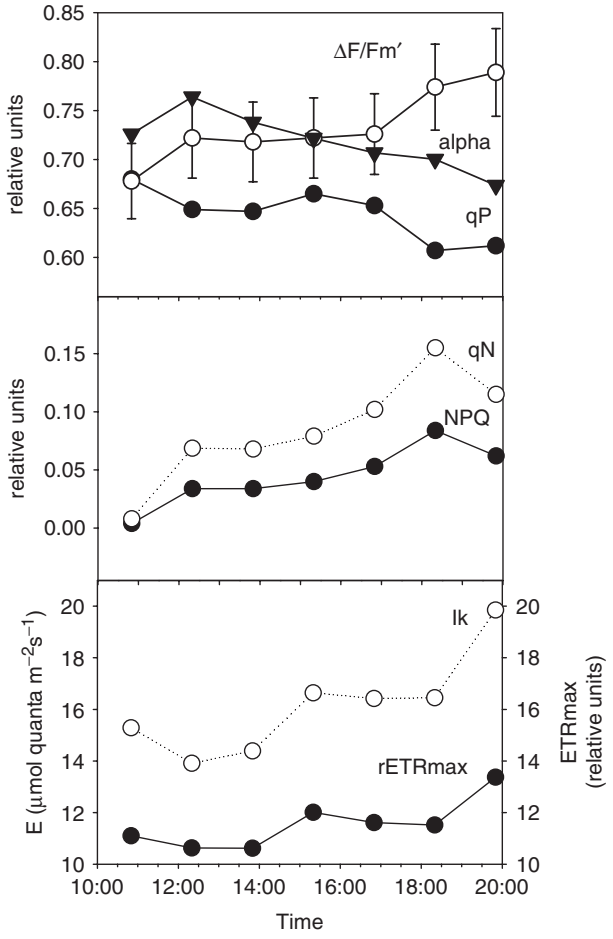

Fig. 4. Photosynthetic parameters of *Ulva expansa* measured *in situ* at 97 m depth on Penguin Bank, Hawai'i between 10:50 and 19:50.

indicate it unlikely that ΔF/Fm′ declined after the onset of darkness. Similarly, true changes in other parameters are most likely in line with estimated values or nil.

Discussion

Apparent depth limits of selected macroalgae

Insufficient irradiance is the most widely accepted factor defining the (lower) depth limit of photolithotrophy. However, the premise that photosynthesis cannot be supported at depths where light is < 1% surface irradiance is not supported by our data nor is it supported in the literature (Larkum *et al.*, 1967; Lüning & Dring, 1979). The crustose coralline alga reported at 268 m by Littler *et al.* (1986) experienced 0.0005% surface irradiance, while in this study the crustose Corallinales at 201 m received only 0.01% surface irradiance. Table 1 lists eight of 12 species in this study at depths where the incident irradiance is less than 1% of surface irradiance (Penguin Bank, 116 m depth, Kd = 0.0395); a range of other deepwater algae are also listed that discredit this premise. The clarity of water at Penguin Bank was greater than that at the Bahamas (Kd = 0.056, Littler *et al.*, 1985) and at Enewetak Atoll (Kd = 0.047, Hillis-Colinvaux, 1986), which are among the clearest waters reported in the literature. The annual photon dose of Corallinales in this study (1.6 mol m^{-2}) is at the upper end of the range reported for encrusting coralline species (4.7 to 0.005 mol m^{-2}, Table 1), which is far less than that of other foliose species. This algal group is

clearly able to survive in an environment with a very low annual photon dose.

Hillis-Colinvaux (1986) and Markager & Sand-Jensen (1992) suggested that at great depths only algae with simple morphologies that are entirely photosynthetic, such as crusts, filaments or unicells are capable of survival. Our observations support this. However Aponte & Ballantine (2001) reported depth limits for several macroalgal species that were relatively shallow (c.f. Littler *et al*., 1985, 1986), and suggested this was in part due to elevated sedimentation load. Unlike the open ocean seamount examined in the Bahamas by Littler *et al*. (1986), our study site on the 'Third Finger' of Penguin Bank has sand on most surfaces except rocky outcrops. This sand is likely to occasionally smother benthic algae, thereby limiting the amount of available light and effectively decreasing the annual photon dose received by these algae. Smothering by loose substratum may help explain why our reported lower depth limits are shallower than those reported for similar or the same species elsewhere.

While quantitative data describing pigment composition and concentration, and photosynthetic absorptance of the algae examined in this study are lacking, thalli of *U. expansa* at 88 to 102 m depth were a rich dark green suggesting a strategy of increasing chlorophyll concentration to increase light absorption. However, this strategy is not necessarily adopted widely; Rodrigues *et al*. (2000) reported an alternate strategy of optimizing light absorption at low-light intensities without increasing pigment content. The composition of pigments of deep-water species is likely to provide more insight into the physiological mechanisms employed by these species and may form the basis of a future study.

In situ *fluorescence: quenching processes*

As many of the species examined in this study are also found in shallow high-irradiance environments, and as many demonstrate a capacity to tolerate irradiance at intensities in excess of that generally experienced at depth, it seems plausible that the deep-water individuals are derived from individuals from shallower waters. Of these, algae that are strongly low-light adapted would be less able to cope with high irradiance exposure (e.g. RLCs) than algae that have retained a capacity to cope with higher light environments.

RLCs perform several functions. An RLC will both expose an alga to a defined irradiance treatment, and will measure the response of the photosystem to that irradiance treatment in terms of the efficiency of photochemical energy conversion (i.e. $\Delta F/Fm'$). By examining the proportion of photochemical (qP) and non-photochemical quenching (qN) evident at the end of an RLC one can determine the extent to which the alga has been able to cope with the excess irradiance, for example elevated qN values indicate the induction of photoprotective processes. Of the green algae examined in this study (*Ulva expansa* and *Microdictyon umbilicatum*), qN was not developed, even at their depth limit, although qP declined markedly at depths below around 100 m. While the high irradiance of the RLC caused a decline in the capacity of the alga to undertake photochemical activity, there was little capacity to activate any photoprotective mechanisms that would have been evident with an increase in qN. Therefore the green algae examined were poorly suited to coping with irradiances in excess of that experienced at depth, and this was particularly evident at depths exceeding 100 m.

The red and brown algae responded differently to RLCs, with an increase in qN and a decline in qP with increasing depth. These algae (including the crustose Corallinales) were able to activate non-photochemical mechanisms to ameliorate the presumably damaging effect of the irradiance treatment. Notwithstanding this increase in photoprotective capacity with depth, there was also a decline in qP which indicates a decline in the capacity to undertake photosynthesis. These algae would appear to be better suited to coping with irradiance in excess of that encountered at depth, and in this sense appear to have retained a photoprotective capacity that is apparently lost by the deeper green algae. This capacity may enable deeper algae to cope with the rapid dark-light transition experienced when a smothering layer of sediment is removed. The ability to tolerate rapid changes in insolation may be an important characteristic that defines the lower depth limit of benthic phototrophs. Whether the lack of photoprotection in deepwater green algae is a consequence of these algae being a genotypically distinct population relative to shallower conspecifics, or whether they are simply exhibiting phenotypic plasticity in response to high irradiance is unclear. Molecular studies on algal material collected in this study may help address this question.

In situ *fluorescence: Ek and electron transport rates*

For the algae examined in this study, fluorescence-derived Ek was generally greater than the maximum ambient irradiance possibly encountered at the depth where the *in situ* measurements were made. In particular, an Ek value of $4.2 \pm 2\,\mu mol\,m^{-2}\,s^{-1}$ irradiance for the encrusting coralline algae at 201 m depth is 20 times greater

than the maximum predicted ambient irradiance at this depth ($0.2\,\mu mol\,m^{-2}\,s^{-1}$), and is 420 times greater than the irradiance during the measurement ($0.01\,\mu mol\,m^{-2}\,s^{-1}$). At depths shallower than 201 m, the Ek of encrusting corallines tends to more closely approximate the maximum predicted ambient irradiance (< 1 to 3 times). Similarly, values of (oxygen-evolution derived) Ek reported elsewhere at depths to 20 m were within an order of magnitude of the ambient irradiance at the depth of sampling, and Ek declined with increasing depth (Johansson & Snoeijs, 2002). In contrast, the coralline alga *Hydrolithon onkodes* (Heydrich) D. Penrose & Woelkerling in high-light waters had (oxygen-evolution derived) Ek values that were more than an order of magnitude less than the maximum irradiance at the location of measurement (Payri *et al*., 2001). Our data suggest that deep-water algae have a capacity for conducting electron transport at rates in excess of those ever likely to be encountered naturally in deeper waters. *In situ* gas exchange measurements would complement our fluorescence-derived data by providing a snapshot of the carbon balance of the algae. As fluorescence-derived ETR generally overestimates photosynthetic activity during high irradiances relative to gas-exchange derived estimates (Longstaff *et al*., 2002), values of Ek of relatively shallow algae in this study may over-estimate the irradiance where net carbon gain reaches a maximum rate. However, values of Ek derived from either fluorescence or gas-exchange methods will be similar at low irradiances, so values of Ek for the deep Corallinales most closely represent the maximum irradiance for net carbon gain.

Values for absorptance can improve our understanding of the strategies adopted by an alga to survive a low-light environment (in terms of increasing either photosynthetic pigment concentration or photon-conversion efficiency), and are used in calculating electron transport rate, which is the product of $\Delta F/Fm'$, E, absorptance and the proportion of energy distributed between PSI and PSII (Genty *et al*., 1989).

Effective quantum yield of chlorophyll fluorescence

With increasing depth, photosynthetic efficiency ($\Delta F/Fm'$) of the species examined remained unchanged, with averages value for encrusting coralline algae of 0.60 and green algae of 0.69. During the diel deployment, $\Delta F/Fm'$ of *U. expansa* at 106 m was steady at 0.720 from 10:30 to dusk, with an increase to 0.774 (18:20) and 0.779 (19:20) after dark (Fig. 4). The small variation and high values indicate minimal photoprotection during the day, while the possible decline in alpha after dark suggests a slight reduction in photosynthetic efficiency in response to a light treatment (RLC). By down-regulating photoprotection during the night, the algae can reallocate resources elsewhere.

Stable isotope ratios

Of the species examined in this study, only the $\delta^{15}N$ and C : N of *M. umbilicatum* declined with increasing depth (Table 2). A decline in $\delta^{15}N$ with depth could be attributed to a decline in nitrate uptake with increasing depth, as the diffusive influx of ammonium is energetically inexpensive relative to active uptake of nitrate (Turpin, 1991). However, no other species demonstrated a depth-dependent change in $\delta^{15}N$, suggesting generally minimal N isotope discrimination in waters from ~80 to 140 m on Penguin Bank. A decline in C : N reflects increasing N uptake and/or a decline in C fixation, of which the latter is most easily attributed to increasing depth. Alternatively, the lower C : N could be due to excretions of cryptic animals within the thallus matrix.

With increasing depth, diffusion of inorganic carbon plays a larger role in inorganic carbon supply. As diffusion is subject to preferential fractionation where the smaller ^{12}C atoms diffuse more readily than the larger ^{13}C atoms, the proportion of ^{12}C diffusing into an alga would increase, leading to more negative $\delta^{13}C$ values. This was not evident for any of the species examined, although differences in $\delta^{13}C$ were evident between species. The absence of any depth-related decline in $\delta^{13}C$ of *H. distorta* and the encrusting Corallinales, and values more positive than surrounding seawater at −9.2‰, was likely due to incomplete removal of inorganic carbon during the acidification process prior to isotopic analysis; similar values for acid-treated Corallinales are reported (Raven *et al*., 2002). The majority of algae examined had $\delta^{13}C$ values within the range of −10 to −30‰, eliminating the exclusive use of CO_2 or HCO_3^- (Raven *et al*., 2002). *Cryptonemia* sp. had the most negative value (−35.38‰), and could be the only species relying solely on diffusive supply of CO_2 to RuBisCO.

Δ represents the difference between the $\delta^{13}C$ values of the sample measured and the source of (inorganic) carbon used in photosynthesis, and is generally calculated assuming this inorganic carbon is directly sourced from seawater. Of algae with Δ values in excess of 20‰, the CO_2 supply is restricted to diffusive influx (Maberly *et al*., 1992). In this study, only *Osmundaria* sp., and *Cryptonemia* sp. satisfied this criterion, while *S. macrodontum* had Δ values from 12 to 20‰. Of the former two species, 89 and 96% respectively of the limitation of the rate of photosynthesis can be

(approximately) attributed to carboxylation rather than diffusion (Maberly *et al*., 1992). This suggests these algae were light-limited and the diffusive flux across the boundary layer and uptake were sufficient to meet the carbon fixation demands; the flux was not limiting because the demand for carbon was very low. This is supported by (i) our observations of low irradiances and currents at the site of sampling occasionally exceeding $1\,m\,s^{-1}$, and (ii) evidence that restricting the supply of inorganic carbon to an alga leads to recycling of effluxed ^{13}C-enriched C resulting in less negative $\delta^{13}C$ values (Maberly *et al*., 1992). $\delta^{13}C$ values of algae collected in this study were generally within the range of $\delta^{13}C$ reported for marine macroalgae of the same genus collected from (presumably shallower) locations (Raven *et al*., 2002), although $\delta^{13}C$ of *U. expansa*, *C. mamillosum*, and *Osmundaria* sp. exceeded published values by 1.4, 3.6 and 7.6‰ respectively (Raven *et al*., 1995), reflecting their deeper habit.

The inorganic carbon pump (that aids uptake of HCO_3^- and is used by algae with $\Delta < 20‰$) may energetically be less efficient in low-light environments as it operates against a light-independent leak (Raven *et al*., 1995). In comparison, the influx of CO_2 by diffusion (used by algae with $\Delta > 20‰$) has a photorespiratory cost that increases with irradiance (and can better be met in shallower waters). This difference in energetic costs pertaining to the two mechanisms of inorganic carbon uptake affirms that uptake of CO_2 by diffusion will be more prevalent in deeper algae. This is supported, in part, by the results of this study.

Conclusion

In situ measurements of variable fluorescence of marine macroalgae at the limit of their distribution have provided insight into how different species cope with irradiance in excess of that normally encountered, which in turn elucidates mechanisms employed by these algae which aid their survival in an extremely low-light environment. The *in situ* deployment of a logging fluorometer showed very small changes in $\Delta F/Fm'$ after sunset, and minimal activation of photoprotective processes by the chlorophyte *Ulva expansa* at 107 m. We suggest that *in situ* measurements offer significant advantages over ship-based *in vivo* experiments in terms of detecting subtleties in the physiological responses to irradiance of deep-water macroalgae. As the fluorescence results show, algae at these depths were photosynthetically active. Indeed, the annual photon dose received by comparable marine macroalgae at other locations suggests that the apparent depth limits observed in this study are not entirely defined by water clarity and annual irradiance budgets. Rather, an additional factor such as a reduced annual irradiance dose due to smothering may prevent algal survival and lead to shallower lower depth limits that contrast with limits obtained from locations where that factor is absent.

Acknowledgements

The authors thank Terri Rust and Frank Sansone for their help in sample analysis, the captain and crew of the R/V Ka'imikai-o-Kanaloa, and in particular the Hawai'i Undersea Research Laboratory (HURL) submersible operations team for their support, especially the submersible pilots, Terry Kerby and Max Cremer. We thank the Australian Antarctic Division for the loan of the submersible fluorometers. JWR was supported in 2006 by an Australian Academy of Science travel grant. This study corresponds to Smithsonian Marine Station contribution 729. This study was funded by a grant from the NOAA Undersea Research Program to KJM and JWR. The authors acknowledge helpful comments from anonymous reviewers. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

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