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The use of pulse amplitude modulated fluorometry to determine fine-scale temporal and spatial variation of in situ photosynthetic activity within an *Isoetes*-dominated canopy

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Abstract

We used in situ pulse amplitude modulated (PAM) fluorometry to investigate photosynthetic activity of *Isoetes alpinus* (Kirk) in submerged meadows of Lake Wanaka, New Zealand. We examined diel and depth-related variability in the quantum yield of photosystem II (PSII) under varying ambient light, as well as variability due to self-shading effects within the *I. alpinus* canopy and inter-leaf differences. We also determined the utility of short-term (minutes) irradiance versus yield estimates in assessing longer-term responses to fluctuating light. Results showed that under natural lighting, PSII yield was highly variable within the plant canopy for a given irradiance incident to that canopy, ranging from 0.02 to 0.80. This variability could be explained by relating PSII yield to irradiance within the canopy measured using a miniature scalar irradiance sensor. For individual plants, yield and irradiance were closely related, and quantitatively similar yield–irradiance (Y–E) curves were obtained for plants from a given depth by experiments using diel, short-term (seconds) and in-canopy irradiance changes. Maximum fluorescence yield consistently averaged 0.55–0.65 and the light saturation parameter ($E_k(Y)$) at 3 m depth ranged from 189 to 247. There were water depth-related differences between mean responses of the *I. alpinus* canopy; individual leaves from the maximum and minimum growth depths (7 and 3 m) showed ranges of ($E_k(Y)$) of 159–228 and 109–151 μmol m^{-2} s^{-1}, respectively. Irradiance alone is the dominant determinant of PSII activity in *I. alpinus* in Lake Wanaka, and accurate description of light within a canopy is a pre-requisite to estimating community photosynthesis. This, combined with a comprehensive assessment of the variability of individual plant responses to irradiance, by depth, may provide a sound basis for modelling community activity. We conclude that, by its ability to obtain fine-scale activity measurements rapidly, and with no enclosure or disturbance effects, PAM fluorometry offers new insights to the activity of specific plants and parts of plants in complex submerged canopies. It

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remains to be determined, however, whether PAM-determined quantum yield of PSII in these plants can be directly related to carbon fixation.

Keywords: Isoetes alpinus (Kirk); PAM fluorometry; Irradiance; Quantum yield; Photosynthesis; Lake Wanaka

1. Introduction

The varied ecological roles that submerged macrophytes play within lakes, as primary producers, as periphyton, invertebrate and vertebrate habitats (Sculthorpe, 1971) and in mediating sediment-water interactions (Scheffer, 1998) have led to the development of many models of growth (Carr et al., 1997; Zimmerman et al., 1994). In order to model growth, an understanding of community photosynthesis is required, yet macrophyte communities are, however, an optically complex matrix of photosynthetic and non-photosynthetic tissues (Krause-Jensen and Sand-Jensen, 1998), and modelling photosynthesis in such a system is not straightforward. Typically, macrophyte productivity models use optical models to describe light within the canopy, with distinct attenuation coefficients for the water column and the macrophyte bed (e.g. Schwarz and Hawes, 1996; Carr et al., 1997; Calado and Duarte, 2000). A mathematical formulation relating light to photosynthesis (*P–E* response) is then used to estimate photosynthetic rate. Usually, photosynthetic parameters are estimated from laboratory incubations at a range of light intensities, and assumptions are made regarding the temporal and spatial (in three dimensions) constancy of these parameters (Carr et al., 1997). In situ measurements of photosynthesis are logistically difficult within macrophyte communities, and few attempts to check the assumptions or detailed predictions of production models have been made.

Recent developments in the use of pulse amplitude modulated (PAM) fluorometry with submerged plants in situ has enabled measurement of photosynthetic activity to be made over finely resolved spatial and temporal ranges (e.g. Hanelt et al., 1993, 2002; Beer and Björk, 2000). There is also strong evidence to suggest that yields measured using fluorescence techniques accurately reflect the photosynthetic performance of plants, except at very high irradiance (Hanelt et al., 1995; Beer et al., 1998; Beer and Björk, 2000). The ability to measure photosynthetic activity on centimetre scales, in plants not enclosed in chambers and subject to ambient irradiance now allows testing of some of the assumptions frequently used in estimating photosynthesis in submerged plant canopies. In this paper, we specifically address these issues, by testing the following hypotheses:

1. That under a given irradiance, the photosynthetic activity of plants of the same species, from any depth, will be similar.
2. That diel cycles of irradiance elicit diel cycles of photosynthesis that are related only to irradiance.
3. That regardless of position within a canopy, the relationships between photosynthetic activity and irradiance will be the same for a given species.
4. That the activity of plants over time within a canopy varies according to the distribution of irradiance within the canopy.

To investigate these hypotheses, we carried out a series of measurements of photosynthetic activity in the quillwort *Isoetes alpinus* (Kirk) in Lake Wanaka, on the South Island of New Zealand.

2. Methods

2.1. Study sites

Lake Wanaka (445°S, 169°E) fills a glacially excavated valley in the Southern Alps of the South Island of New Zealand. It is a large lake, with an area of 180 km^2 and a maximum depth of 311 m. The water is generally clear, with secchi disc depths of 9–19 m (Livingston et al., 1986) and this supports a deep growing (50 m), predominantly native submerged vegetation (Clayton et al., 1986). The quillwort *I. alpinus* forms a distinct depth band of vegetation in this lake, reaching from just below low water level to approximately 8 m below median level. Within this band, *I. alpinus* often forms a thick canopy, growing up to 20 cm tall, through which other native plants, particularly *Myriophyllum propinquum*, *M. triphyllum* and *Potamogeton cheesemanii*, grow in places.

Two study sites were established in the lake, at Beacon Point and Ruby Island. Beacon Point (BP 44°39.6′S, 169°06.9′E) is exposed to wind waves from the prevailing NW winds, whereas Ruby Island (RI 44°41.1′S, 169°05.6′E) is more sheltered.

2.2. Experimental procedures

Three series of observations were undertaken, diel measurements of photosynthetic activity and irradiance, short-term photosynthesis–irradiance (*P–E*) curves for plants from a range of depths, and measurements of irradiance and photosynthetic yield within the canopy.

Photosynthetic activity was estimated as variable fluorescence using a Walz Diving PAM (Walz, Germany). This instrument is fully submersible and allows measurement of variable fluorescence in situ, with centimetre spatial resolution, and repeated measurements within a few seconds. Variable fluorescence is a property of photosystem II (PSII) which can be used to estimate photosynthetic activity (Schreiber et al., 1986). The system measures the fluorescence of chlorophyll under ambient irradiance (F_s), and during application of a short pulse of saturating white light (maximum fluorescence F'_m). The difference in fluorescence ($F'_m - F_s$) is called the variable fluorescence (ΔF). The ratio of variable fluorescence to maximum fluorescence ($\Delta F/F'_m$) is the effective quantum yield (Y) of the plant under prevailing irradiance condition (Hanelt et al., 1993). Y is normally at its highest in non-inhibited material under dark-acclimated conditions and tends to decline proportionately to the extent to which photosynthesis is light saturated. The shapes of genuine *P–E* relationships can be approximated as relative electron transport rate (RETR), the product of PSII yield and irradiance.

2.3. Diel measurements

Diel measurements were made at Ruby Island in March 2001. Surface irradiance was measured at 5 min intervals over the course of the day using a calibrated LiCor 190 PAR

sensor recording onto a LiCor Li1000 datalogger. At 1 or 1.5 h intervals, SCUBA divers made multiple measurements of quantum yield of *I. alpinus* plants at 4 m depth, under ambient conditions using the diving PAM. During these measurements, leaves close to the top of the *I. alpinus* canopy were sampled haphazardly with respect to leaf orientation. In addition, at each sampling interval, 15 leaves were dark acclimated for 12–15 min in a leaf clip before a measurement of maximum quantum yield was taken. The extent to which dark-acclimated yield returns to the early morning value is a measure of the extent to which any depression of yield is due to dynamic photoinhibition rather than longer-term acclimation responses (Hanelt et al., 1993, 1995). Underwater irradiance was measured by SCUBA divers using a calibrated LiCor Li192 cosine corrected sensor connected to a LiCor Li189 meter housed in a waterproof box.

In addition, in March 2002, at Beacon Point, we determined the diel response of individual leaves. To undertake this leaves, still attached to the main body of the plant, were secured in a clamp on the lake floor at 5 m depth. The sensing fibre of the diving PAM was directed at the leaf surface, taking care to minimise shading by the fibre. The diving PAM was then programmed to make a fluorescence measurement every 15 min over a 24 h period. Ambient irradiance was measured simultaneously using the diving PAM irradiance sensor, which had been previously calibrated against the LiCor sensor at the working depth.

2.4. Within canopy measurements

In March 2002, at Beacon Point, SCUBA divers used the diving PAM to measure irradiance and quantum yield at fine spatial resolution within the *I. alpinus* canopy at 3, 5 and 7 m depth. At each depth, a series of spot measurements were made of quantum yield and irradiance at three leaf positions; close to the surface of the canopy, midway through the canopy, and close to the canopy base. As far as possible, the measurements of irradiance and yield were made at the same location, and this gave a broad distribution of irradiance within a given depth. Care was taken to disturb the light field within the canopy as little as possible during measurements. The diving PAM irradiance sensor head is normally cosine corrected but, for this experiment, was replaced with a spherical collector. The spherical collector was constructed by dipping the end of the sensing optical fibre in a mixture of clear epoxy resin and titanium dioxide powder several times until a diffusing sphere was built up. The sensor, held over a black matt fabric to avoid upwelling reflected light, was calibrated against a LiCor Li192 sensor at the sampling depth.

2.5. Controlled light measurements

In March 2002, in order to further examine the response of individual leaves to varying irradiance, we used the rapid light curve (RLC) function of the diving PAM. In this mode, the instrument measures quantum yield at a series of nine irradiances from dark to a pre-programmed level. Actinic light for this series was provided by the internal halogen lamp of the diving PAM, and acclimation to each intensity was for 15 s only. Curves were recorded by divers, and the only disturbance to plants was the placing of the leaf clip onto the leaves. As the emission of the internal diving PAM lamp is not stable over time, care was taken to check irradiance regularly with the calibrated light sensor.

2.6. Statistical treatment of data

Where necessary, replicated measurements were compared using the analysis of variance module of Statistica (Statsoft Inc., Tulsa, OK, USA), with post hoc testing using the Tukey method. Significance levels were set at $P < 0.05$. Curves were fitted to yield–irradiance (Y–E) data to allow estimation of key parameters. The curve fitted was a rearrangement of a curve from Smith (1936), which related photosynthesis P to irradiance (E_d) as

$$P = P_{max} \frac{E_d}{(E_d^2 + E_k^2)^{0.5}} \quad (1)$$

where P_{max} is light saturated photosynthesis and E_k is the light saturation parameter. Often PAM-derived data are fitted to such curves using the RETR described earlier. However, deriving RETR, when Y and E_d may both contain measurement error, reduces the ability to assess variance in Y. We, therefore, derived an expression from Eq. (1) linking Y and E_d. This was achieved by assuming that a constant relationship between true quantum yield of photosynthesis (ϕ) and Y; an identical assumption is, of course, implicit in calculation of RETR as the product of Y and E_d. Based on this assumption, we modified Eq. (1) to describe Y in terms of E'_k (E_k derived from yield measurements) and a new parameter Y_{max} (the maximum yield under experimental conditions)

$$Y = Y_{max} \frac{E'_k}{(E'^2_k + E_d^2)^{0.5}} \quad (2)$$

The assumption that Y approximates ϕ is, however, fundamental to this argument and this assumption is supported by developmental work with PAM fluorometry (Schreiber et al., 1986) though, as discussed earlier, at very high irradiance it may break down (Geel et al., 1997). In our experiments, we avoided very high irradiance and thus we hoped that good fits to the relationship could be obtained. The value of Eq. (2) is that, if satisfactory fits can be obtained, Y–E curves can be compared using the ecologically meaningful and conceptually simple coefficients Y_{max} and E'_k and direct estimates of mean and error of both parameters can be made. Parameter estimation was undertaken using the least-squares curve fitting module of Sigmaplot 2001 (SPSS Inc., Chicago, IL, USA) which gave estimates and standard errors of Y_{max} and E'_k.

3. Results

3.1. Diel observations

The first series of diel observations, where plants were sampled randomly with no conscious bias for leaf orientation or position in the top of the canopy, a large range of yields were observed at each sampling time (Fig. 1). There was a decline in median yield as incident irradiance increased, reaching a minimum in mid afternoon. ANOVA identified the yields taken between 13:30 and 17:30 h as being lower than those at all other times, with the 19:00 h yields returning to morning values. Dark-adapted yield also decreased slightly during the day, and again ANOVA identified groups of samples, those between 13:30 and

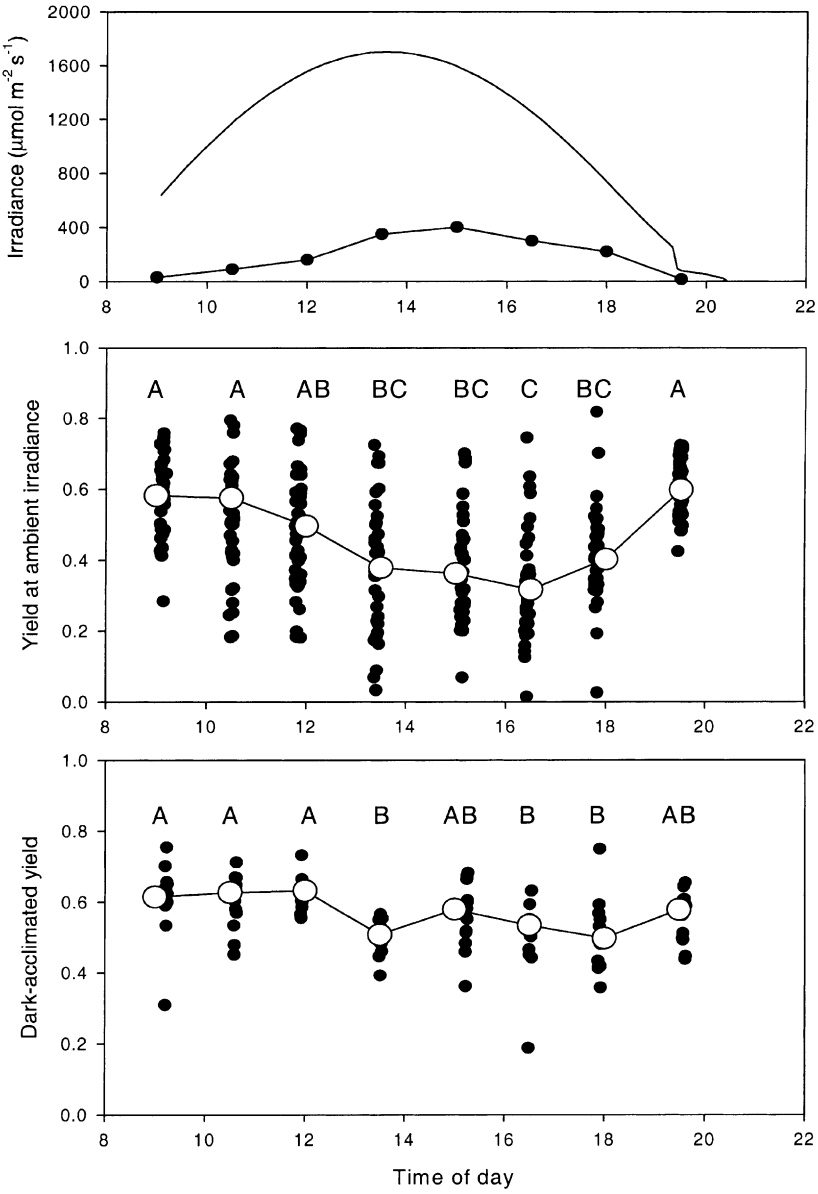

Fig. 1. Diel changes in yield in *I. alpinus* at Ruby Island. The top panel shows the incident irradiance (upper curve) and that measured at the sampling depth; the two curves are out of phase as the site has a westerly aspect. The middle panel shows the yield under ambient light, and the lower the yield after dark adaptation. In the lower two panels, black dots are individual records and the open circles are the median values for each interval. Letters in the lower two panels indicate ANOVA groupings.

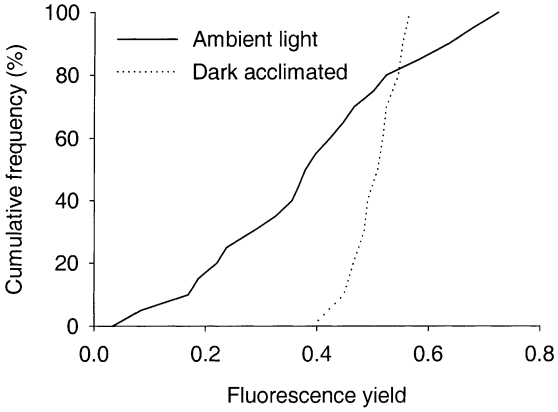

Fig. 2. Cumulative frequency curves for fluorescence yield of *I. alpinus* at 3 m depth in Lake Wanaka at 18:00 h. The curves show yield distributions under ambient light and after 15 min of dark acclimation.

17:30 h tending to be lower than those either before and after. The distribution of yields was much wider in the light than after dark acclimation, with the 25–75 percentiles ranging between 0.24 and 0.50, and 0.48 and 0.53, respectively (Fig. 2).

When plotted against irradiance immediately above the canopy, median yield within the upper canopy showed a general decrease with increasing irradiance, which was reversed in the afternoon (Fig. 3). However, the enormous variability in yield values seen amongst the leaves sampled on each occasion made it impossible to identify clear patterns. Given the variability seen in our first diel experiment, the second was designed to follow individual

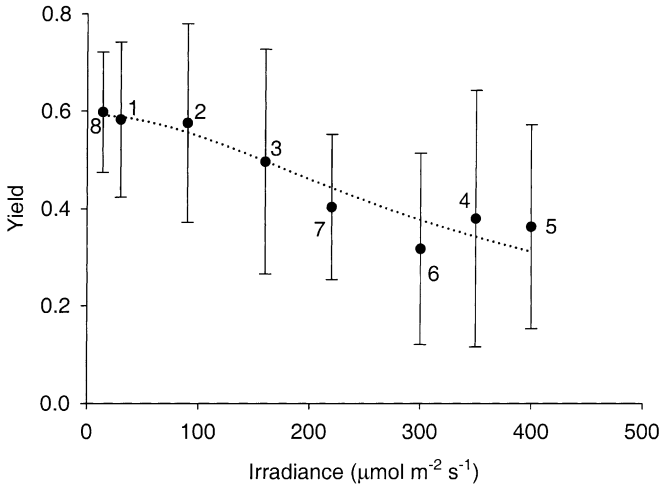

Fig. 3. Median and interquartile range of yield of *I. alpinus* under ambient irradiance during the diel experiment at Ruby Island. Median values are numbered in time sequence. The curve fitted to the median values is the yield curve described in Eq. (2). Yield–irradiance parameters are given in Table 1.

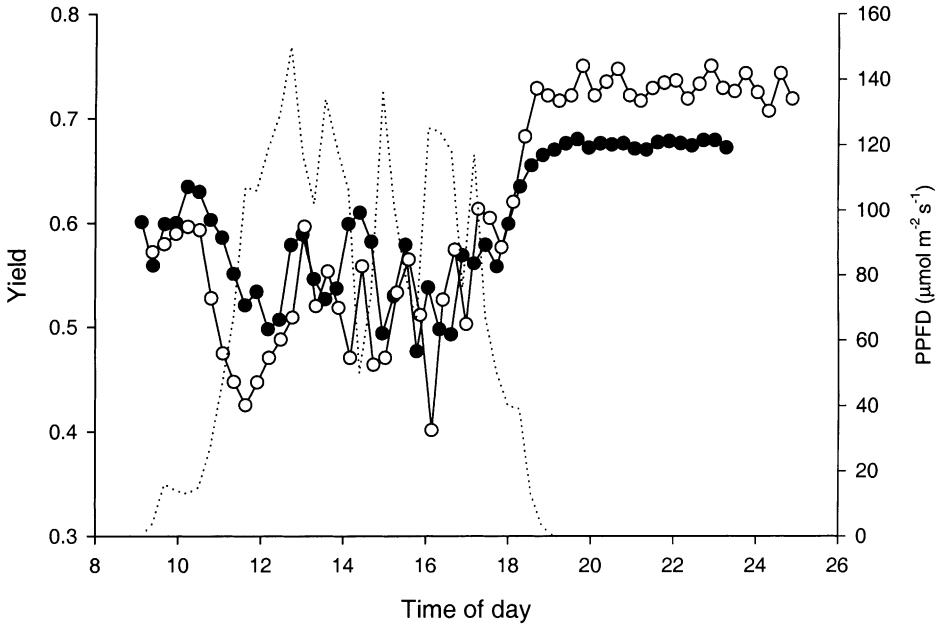

Fig. 4. Diel variation in yield of two leaves of *I. alpinus* exposed to ambient irradiance at 5 m depth in Lake Wanaka. Irradiance is indicated by the broken line.

leaves through a daily irradiance cycle. Under these conditions, there was a clear daily response of leaf yield, which closely mirrored the fluctuations in irradiance on this day of patchy cloud cover (Figs. 4 and 5). Examination of raw records of the two fluorescence parameters showed that most of the variation in yield was due to down-regulation of F'_m at high irradiance, and that this down-regulation was reversed rapidly in the evening (Fig. 6). A feature seen in both plants was a drop in F'_m shortly after dawn, which resulted in an unusually low yield.

3.2. Within canopy measurements

Relationships between fluorescence yield within the canopy and irradiance at that point also showed good fits to the *Y–E* formulation of Eq. (2) (Fig. 7, Table 1). We found that samples taken from 7, 5 and 3 m tended to show similar shaped curves (Table 1) and a single curve provided a good model of all samples from the three depths. The irradiances in the data from the three depths overlapped, since it was universally low at the base of the canopy, but there will have been some degree of biasing of the curve by shallow samples at high irradiance, because the highest irradiances were only recorded at 3 m. Based on within canopy sampling, average values of 180 $\mu mol\, m^{-2}\, s^{-1}$ for E'_k and 0.58 for Y_{max} were obtained (Table 1). Analysed separately by depth, there was a tendency for the deeper plants to show higher Y_{max} and lower E'_k, but the extensive overlap between errors on these estimates suggest that these differences are not significant (Table 1).

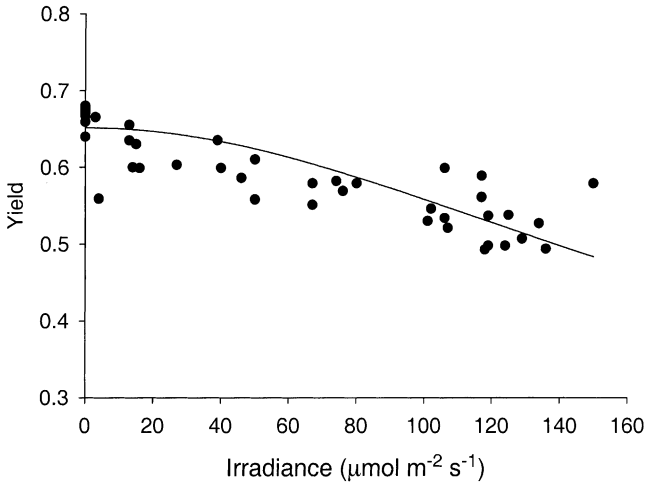

Fig. 5. The relationship between irradiance and yield measured at 15 min intervals over the course of a day in a single leaf of *I. alpinus* at Beacon Point. Yield–irradiance parameters are given in Table 1.

3.3. Controlled light measurements

Rapid light curves, which measure the fluorescence yield–irradiance response at a given state of photoacclimation, were undertaken at close to midday on plants from deep (7 m)

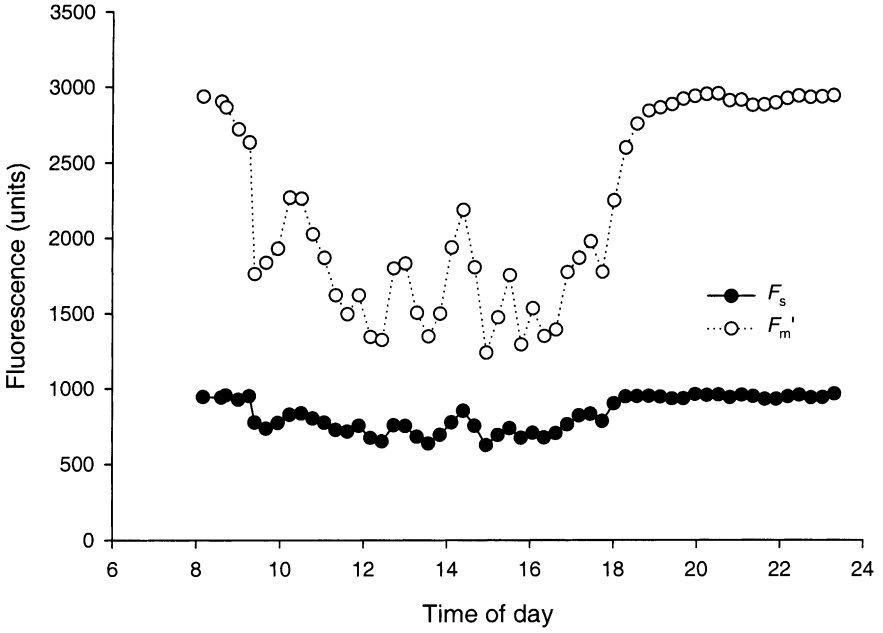

Fig. 6. Time series of F_t and F'_m from one of the *I. alpinus* plants illustrated in Fig. 6.

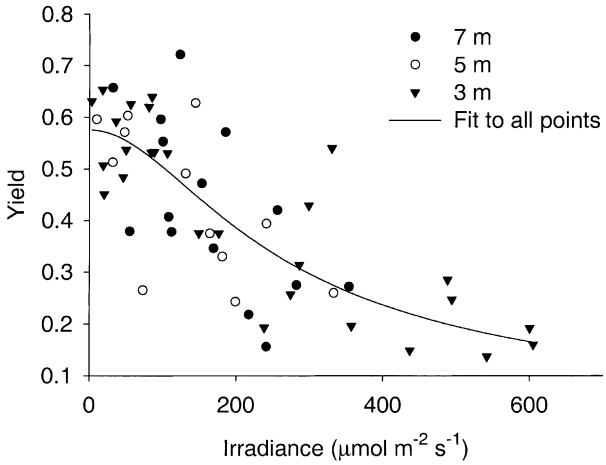

Fig. 7. Yield–irradiance curve for *I. alpinus* from three depths at Beacon Point in Lake Wanaka. Measurements of irradiance and yield were made through the canopy at each depth. The curve fitted is for the entire dataset. Yield–irradiance parameters are given in Table 1.

and shallow (2.5 m) at Beacon Point. Good fits of the *Y*–*E* curve (Eq. (2)) were obtained when the data from the three curves from each depth were considered together (Fig. 8, Table 1). Parameters derived from these pooled data curves were sufficiently different, with no overlap of error bars, as to imply genuine differences. The shallow plants had lower maximum fluorescence yield and a higher E'_k than the deep plants. When the curves for the individual plants were parameterised separately, this difference remained, but was not so clear cut (Table 2). Values of Y_{max} from the two depths overlapped, and E'_k came close to overlapping. While mean values of parameters varied between the depths, the extent of variation between leaves from the same depth was large, and indicated that even across the 3–7 m depth range, leaves with similar *Y*–*E* relationships could be found.

Table 1
Computed values of the yield–irradiance parameters (mean ± S.E.) from the different series of observations

Experiment and location	Depth (m)	Y_{max}	E'_k	N	r^2	P
Diel 1, Ruby Island	3	0.59 ± 0.01	247 ± 29	7	0.89	0.0004
Diel 2, Beacon Point	5	0.65 ± 0.01	166 ± 9	54	0.72	<0.0001
Within canopy, Beacon Point	All data	0.58 ± 0.03	180 ± 23	54	0.59	<0.0001
	3	0.58 ± 0.03	189 ± 29	26	0.74	<0.0001
	5	0.55 ± 0.06	179 ± 59	11	0.41	0.02
	7	0.59 ± 0.09	164 ± 60	14	0.40	0.01
RLC Beacon Point	3	0.58 ± 0.01	193 ± 18	27	0.83	<0.0001
	7	0.65 ± 0.01	117 ± 9	27	0.90	<0.0001

RLC indicates the rapid light curves. In these analyses, three curves, each of nine points, were made at each depth. Observations at Ruby Island were undertaken in February 2001, those at Beacon Point in February 2002. Y_{max}: maximum photosynthetic yield (dimensionless); E'_k: irradiance for the onset of saturation of photosynthesis derived from yield measurements (μmol photons m^{-1} s^{-1}).

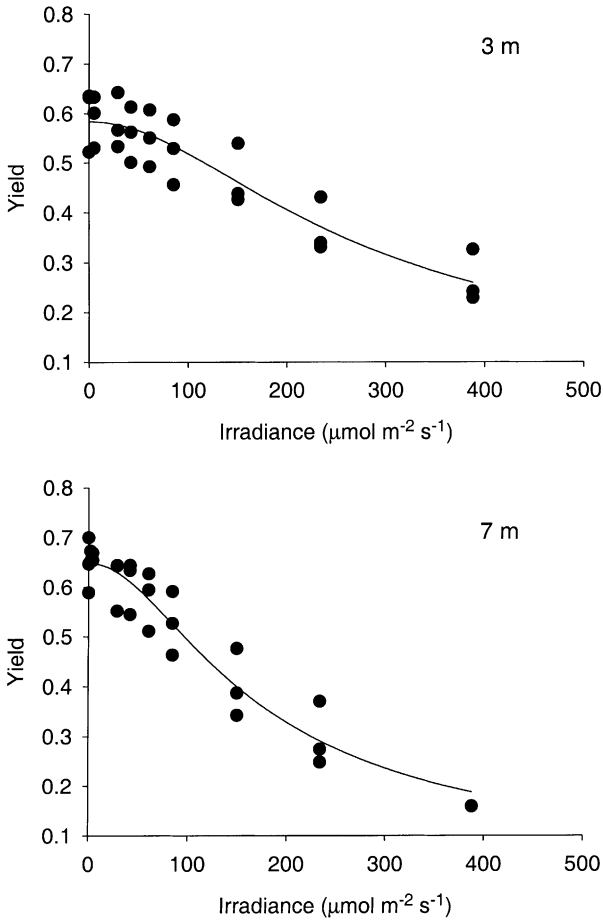

Fig. 8. Short-term yield–irradiance curves for *I. alpinus* from 3 m depth (upper) and 7 m depth (lower) at Beacon Point in Lake Wanaka made under controlled irradiance. The curve fitted in each case is for the entire dataset. Yield–irradiance parameters are given in Table 1.

4. Discussion

In this study, we have used PAM fluorometry to investigate the photoadaptive state of *I. alpinus* under controlled irradiance and with changing ambient irradiance over the course of a day and through a canopy. The ability of PAM methods to take near instantaneous measurements of an uncontained leaf under ambient conditions makes it particularly well suited to this type of investigation. We have used the fluorescence yield parameter (Y) throughout the study as an indicator of saturation status and, to facilitate comparison of responses, have developed an expression relating fluorescence yield to irradiance (Eq. (2)).

Use of Eq. (2), to derive Y_{max} and E'_k values was successful in that highly significant regressions resulted from all experiments. The shape of the relationship is as would be

Table 2
Computed values of the yield–irradiance parameters (mean ± S.E.) from RLCs of three plants at each of two depths

Depth	E'_k	Y_{rnax}
3 m, plant A	159 ± 9	0.59 ± 0.01
3 m, plant B	228 ± 6	0.63 ± 0.00
3 m, plant C	196 ± 9	0.52 ± 0.00
7 m, plant A	109 ± 2	0.58 ± 0.00
7 m, plant B	151 ± 6	0.67 ± 0.01
7 m, plant C	111 ± 4	0.66 ± 0.01

In all cases, the regressions were highly significant ($P < 0.0001$). E'_k irradiance for the onset of saturation of photosynthesis derived from yield measurements (μmol photons m^{-1} s^{-1}); Y_{max}: maximum photosynthetic yield (dimensionless).

expected, with high fluorescence yield at very low irradiance, declining as irradiance increasing to a near linear decline above saturation of PSII. In Eq. (2), E'_k largely drives the curvature of the relationship, while Y_{max} sets the position of the curve. Whether E'_k is synonymous with E_k for photosynthesis depends on whether the factor product of fluorescence yield and irradiance (RETR) to photosynthesis is constant across irradiance. While there is evidence that in some aquatic plants, at very high irradiance, this is not the case (Beer and Björk, 2000), these same authors noted that at low and moderate irradiance there was an excellent agreement, suggesting that E'_k is a likely to be a good surrogate of E_k. Regardless of this, E'_k is a useful parameter for comparing plant responses, since it indicates the irradiance at which PSII becomes light saturated. In analogy to E_k, plants acclimated to low irradiance might be expected to have lower E'_k than plants from high light environments (e.g. Kirk, 1994).

In setting up the study, we erected a series of hypotheses to test assumptions frequently made while estimating photosynthesis in aquatic plant canopies. The first of these was that under a given irradiance, the photosynthetic performance of plants of the same species, from any depth, would be similar. Table 2 indicates that this was not the case, since under identical irradiance regimes, E'_k ranged from 160–230 μmol m^{-2} s^{-1} at 3 m to 110–150 μmol m^{-2} s^{-1} at 7 m depth, and Y_{max} also varied. While there was a tendency for deeper plants to have lower E'_k, the values for the two depths came close to overlapping. We therefore conclude that there is considerable within-depth variation in *Y–E* relationships in *I. alpinus*, and that to model photosynthesis sufficient plants need to be measured to obtain estimates of mean and variance of the key parameters.

PAM fluorometry, and specifically rapid light curves, offers a possibility of rapid measurement of *Y–E* parameters. Can these RLCs substitute for daily time course measurements of parameters? Table 1 indicates that, for the 3 m samples, estimates of both E'_k and Y_{max} by RLC and by single-plant diel or through-canopy measurements were similar, though the RLC values for 7 m were not. Acclimation to changing irradiance is not an instantaneous process, and a series of physiological mechanisms, are involved (Krause and Weis, 1991; Büchel and Wilhelm, 1993). The short period of acclimation time available during a RLC (15 s) is inadequate for full acclimation. That long-term responses are involved is confirmed by the hysteresis seen in the Ruby Island diel experiment, where under high incident

irradiance there was a slight depression of afternoon fluorescence yield s with respect to morning yields at similar irradiance (Figs. 1 and 3). RLC-derived estimates of photosynthetic parameters are further compromised by the fact that the spectrum of the halogen lamp used for these measurements is different to the ambient under water light spectrum, which will affect absorption characteristics and hence photosynthetic performance (Hanelt et al., 2002). The data thus provide only limited support for the use of RLC-derived *Y–E* parameters for modelling photosynthesis within the canopy, and further work in this area is clearly required before the optimum acclimation time is determined. For example, Krokamp et al. (1998) illuminated benthic microbial mats for 2 min prior to measuring light responses.

The second hypothesis in this study was that diel cycles of irradiance elicit diel cycles of photosynthesis that are related only to irradiance. This is again a prerequisite for use of optical models combined with *P–E* relationships to estimate production. In the single-leaf diel investigations this was found to be the case, though in these experiments ambient irradiance was rather low, barely reaching E'_k. In the other diel experiment, ambient irradiance was higher, above E'_k, and we saw some degree of persisting inhibition of photosynthesis during the afternoon. This was also evident in the dark-acclimated fluorescence yield measures, where full recovery of Y_{max} was not achieved within the 15 min (Fig. 1). High light stress, particularly when this includes ultra-violet wavelengths, has previously been shown to have this type of prolonged inhibitory effect on aquatic plants (Bischof et al., 2001). However, in our experiment it was clear that the variability of individual leaf response was sufficiently high to lose the afternoon inhibition within the error bars.

Much of the variance for individual time points in Fig. 1 may have been related to inter-plant or inter-leaf differences in *Y–E* responses, as discussed above, or small-scale variations in irradiance experienced within the canopy. Hypothesis three was that regardless of position within a canopy, the relationships between photosynthetic activity and irradiance will be the same. Fig. 7 and Table 1 indicate that, within the constraints imposed by inherent variability, regardless of depth of collection, or position within the canopy, a single *Y–E* curve described all of the samples at least as well a depth-specific analyses. Part of the reason for this is likely to be that irradiance was attenuated rapidly within the canopy, so almost all measurements made at the base of the canopy are at irradiances well below E'_k, hence *Y* tended towards Y_{max}. Thus, any physiological acclimation to low irradiance in the lower parts of the canopy will not be evident in Fig. 7 but would require specific measurements of *Y–E* relationships for basal parts of leaves.

The final hypothesis we erected was that the activity of plants within a canopy varies according to the distribution of irradiance within the canopy. This combines our previous hypotheses to suggest that knowledge of the distribution of irradiance within a canopy, and of the depth-specific *Y–E* responses of the plant will enable description of whole canopy response. The consistency amongst parameters describing *Y–E* relationships made using a variety of irradiance variations confirm that irradiance was the major driver of fluorescence yield, regardless of depth or position. Inter-depth and inter-leaf variability in *Y–E* parameters was, however, evident, and an understanding of the nature of this variance, and its underlying causes would enhance the understanding of canopy photosynthesis.

Overall, this study has demonstrated the utility of the diving PAM for making measurements of PSII fluorescence yield in freshwater plants, under ambient conditions and with no complications imposed by enclosures. We have also shown that a simple rearrangement of

an established photosynthesis–irradiance curve allows the *Y–E* curves to be parameterised in a meaningful way, to yield parameters analogous to those used in other types of photosynthesis measurements. Such parameterisation has shown that, for *I. alpinus* in Lake Wanaka, there is degree of consistency in apparent *Y–E* relationships between and within depths and over time, but that considerable variability exists between plants. For *Isoetes*, a generalised response, with estimation error, could be applied quite broadly to describe the yield–irradiance relationship. Within the confidence band that this variance dictates, an approximation of the likely photosynthetic performance in a complex *Isoetes* canopy can be derived from the generalised *Y–E* relationships and the irradiance distribution within that canopy.

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