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Modelling photosynthesis in shallow algal production ponds

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Abstract

Shallow ponds with rapidly photosynthesising cyanobacteria or eukaryotic algae are used for growing biotechnology feedstock and have been proposed for biofuel production but a credible model to predict the productivity of a column of phytoplankton in such ponds is lacking. Oxygen electrodes and Pulse Amplitude Modulation (PAM) fluorometer technology were used to measure gross photosynthesis (P_G) *vs*. irradiance (E) curves (P_G *vs*. E curves) in *Chlorella* (chlorophyta), *Dunaliella salina* (chlorophyta) and *Phaeodactylum* (bacillariophyta). P_G *vs*. E curves were fitted to the waiting-in-line function [$P_G = (P_{Gmax} \times E/E_{opt}) \times \exp(1 - E/E_{opt})$]. Attenuation of incident light with depth could then be used to model P_G *vs*. E curves to describe P_G *vs*. depth in pond cultures of uniformly distributed planktonic algae. Respiratory data (by O_2-electrode) allowed net photosynthesis (P_N) of algal ponds to be modelled with depth. Photoinhibition of photosynthesis at the pond surface reduced P_N of the water column. Calculated optimum depths for the algal ponds were: *Phaeodactylum*, 63 mm; *Dunaliella*, 71 mm and *Chlorella*, 87 mm. Irradiance at this depth is ≈ 5 to 10 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). This knowledge can then be used to optimise the pond depth. The total net P_N [μmol(O_2) m^{-2} s^{-1}] were: *Chlorella,* ≈ 12.6 ± 0.76; *Dunaliella,* ≈ 6.5 ± 0.41; *Phaeodactylum* ≈ 6.1 ± 0.35. Snell's and Fresnel's laws were used to correct irradiance for reflection and refraction and thus estimate the time course of P_N over the course of a day taking into account respiration during the day and at night. The optimum P_N of a pond adjusted to be of optimal depth (0.1–0.5 m) should be approximately constant because increasing the cell density will proportionally reduce the optimum depth of the pond and *vice versa*. Net photosynthesis for an optimised pond located at the tropic of Cancer would be [in t(C) ha^{-1} y^{-1}]: *Chlorella*, ≈ 14.1 ± 0.66; *Dunaliella,* ≈ 5.48 ± 0.39; *Phaeodactylum,* ≈ 6.58 ± 0.42 but such calculations do not take weather, such as cloud cover, and temperature, into account.

Additional key words: algal production ponds; *Chlorella; Dunaliella;* electron transport rate; light saturation curves; *Phaeodactylum*; photoinhibition; photosynthesis; photosynthesis *vs*. depth; primary productivity; pulse amplitude modulation fluorometry.

Introduction

Photosynthesis in shallow ponds follows similar principles in terms of photosynthesis to deeper lakes and the ocean, except that to make shallow ponds efficient converters of solar to organic energy the algal concentration has to be very high and stratification has to be absent. Several past studies have addressed the general situation and have integrated total net photosynthesis (P_N) over the water column (Lorentzen 1976, Falkowski 1981, Platt and Sathyendranath 1988, Grobbelaar *et al.* 1990, Sukenik *et al.* 1991, Morel 1991, Bidigare *et al.* 1992, Cullen *et al.* 1993, Behrenfeld and Falkowski 1997). A few studies such as Sukenik *et al.* (1991) have addressed

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Abbreviations: Chl – chlorophyll; E – irradiance 400–700 nm photosynthetic photon flux density (PPFD); E_0 – irradiance at a pond surface, E_{opt} – optimum irradiance; E_x – irradiance at a depth x in a pond; k_L – attenuation constant; PPFD – photosynthetic photon flux density; P_G − gross photosynthesis expressed on an oxygen basis (P_G = rETR/4), P_N – net photosynthesis ($P_N = P_G + R$), R – respiration, rETR – relative electron transport rate; Φ_{PSII} – effective quantum yield.
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the situation at the limit of maximal production, *i.e.*, with optimum combination of concentration of phytoplankton and total pond depth. It turns out that very shallow ponds are optimal systems for production of algal biomass.

By definition, at the compensation depth the total P_N at that depth layer of the pond would be zero because gross photosynthetic carbon fixation would equal losses due to respiration (Grobbelaar *et al.* 1990). The present model applies to a pond that is very shallow such that wind action would result in a homogeneous water column or one gently stirred with a paddlewheel or circular plate (Pasveer-type oval circuit pond: Shimamatsu 1987, 2004; Weissman *et al.* 1988, Grobbelaar *et al.* 1990, Sukenik *et al.* 1991, Zmora and Richmond 2004, Moheimani and Borowitzka 2006a,b; Grobbelaar 2007). Such ponds are used as oxidation ponds in sewage and other wastewater treatment, aquaculture and increasingly in the biotechnology industries and they are commonly thought to have potential for bioenergy production. The value of algae in shallow sewage ponds to provide an aerobic environment for the disposal of sewage has long been recognised and is not controversial (Oswald 1973). The value of shallow algal ponds in the commercial production of high-value products such as β-carotene is also well established (Borowitzka and Borowitzka 1988, Borowitzka 1992, 1999; Grobbelaar 2007): the issue of whether shallow algal ponds could feasibly be used for bioenergy production or be used to achieve significant CO_2 sequestration is more controversial (Weissman *et al.* 1988, Borowitzka 1992, 1999; Sheehan *et al.* 1998, Richmond and Zou 1999, Moheimani and Borowitzka 2006a,b; Antoni *et al.* 2007, Huntley and Redalje 2007, Chisti 2007, 2008a,b; Waltz 2009, Walker 2009, 2010; Larkum 2010, Ritchie 2010). Essentially in an algal production pond the aim is to attempt to sustainably manage an algal bloom but real understanding of algal blooms under such conditions is limited (Smayda 2006).

In a pond, before light has penetrated the water column, irradiance is modified at the air/water interface where light is partially reflected or is refracted depending upon the solar angle (Snell & Fresnel equations; Lorrain *et al.* 1988): the refractive index (n_t) of air at 25°C was taken as 1.00029, freshwater as ≈ 1.3330, seawater (34.3‰) ≈1.3392 and ≈ 1.3577 for 150‰ for *Dunaliella* brine). The transmitted light then decreases approximately exponentially with increasing light path through the pondwater (Lambert's law; Lorrain *et al.* 1988). In the present case, because the pond is assumed to be very shallow, spectral changes due to the water itself have been assumed to be minimal (unlike the case in oceanic seawater and deep clear lakes). A dense growth of any algae in a pond would attenuate the irradiance available to the alga with depth. There would be an upper layer in which the light would be saturating and even photoinhibitory, leading to poor conversion efficiency. Surface photoinhibition is well known to oceanographers and limnologists (Morel 1991, Miller 2006, Falkowski and Raven 2007). In somewhat deeper layers light decreases exponentially to a point where the light regime provides the conditions for efficient energy conversion and maximum photosynthesis. Below this there will be a layer in which the light intensity becomes too low to provide enough photosynthesis to match respiration. The depth at which P_G equals R and hence P_N is zero is called the compensation depth. A uniform pond deeper than the compensation depth would consume more carbon than it produced. This may be a desirable situation in sewage ponds but not in ponds designed to produce an organic carbon product. For deeper ponds, if the algal concentration is reduced then light penetrates further and the zone of efficient photosynthesis is thicker. However, deeper ponds would require high inputs of energy to maintain homogeneity and avoid stratification. Expressed in terms of ambient irradiance, if P_G *vs*. E curve obeys the waiting-in-line equation (*see* Appendix) then photosynthesis would be inhibited by more than 50% in surface layers where irradiance was greater than about 2.5 times the optimum irradiance and inhibited by more than 50% in the depths of a pond where irradiance was less than about 25% of the optimum irradiance. Hence, in a pond there is a zone experiencing from about 0.25 to about 2.5-times the optimum irradiance where the ambient irradiance is favourable for photosynthesis.

In the most efficient ponds the algal concentration is so high that all three layers occur over a few centimetres and efficient stirring would be necessary to maintain optimal production. A paddle-wheel driven Pasveer-type pond described in detail by Shimamatsu (1987, 2004) is the typical engineering solution. In the present study we do not address the practicalities or cost of achieving adequate stirring: we assume that stirring efficiently mixes the algae so that photosynthesis is maintained on average at a high rate and no cells are either severely photoinhibited by excess light or left for long in the deeply shaded depths of the pond. This situation has been addressed in theory (Engqvist and Sjöberg 1980, McBride 1992) and several attempts have been made to integrate total P_N over the water column in more practical situations to estimate the optimum combination of concentration of phytoplankton and total pond depth (Lorentzen 1976, Falkowski 1981, Weissman *et al.* 1988, Platt and Sathyendranath 1988, Grobbelaar *et al.* 1990, Sukenik *et al.* 1991, Morel 1991, Bidigare *et al.* 1992, Cullen *et al.* 1993, Behrenfeld and Falkowski 1997, Grobbelaar 2007). Talling *et al.* (1973) studied very high concentrations of natural populations of cyanobacterial phytoplankton in alkaline lakes and showed that maximum P_N occurred when the alkaline lake had a depth of 0.6 m or less. Kroon *et al.* (1989) modelled P_N in algal ponds and showed that the highest production occurred at the shallowest depth they used in their modelling (0.3 m). Kroon *et al.* (1989) reasoned that ponds shallower than 0.3 m would be unstable and turbid because bottom sediment would be stirred up and they would be difficult

to engineer and manage. On the contrary, Grobbelaar *et al.* (1990) used large Pasveer raceway ponds for growing *Scenedesmus* that were only 150 mm deep. Pasveer ponds used for the commercial production of *Spirulina* have surface areas of up to 5,000 m^2 and a depth of only 0.15 to 0.3 m (Shimamatsu 1987, 2004).

In considering the algal species which could potentially be used in shallow ponds, a wide choice is available. In addition blue-green algae or cyanobacteria (which are not technically algae but photosynthetic prokaryotes) should also be considered. Green algae (chlorophyte divisions of eukaryotic algae), charophytes and archeogoniophytes, which include vascular and non-vascular land plants, have the shared characteristic of having chloroplasts containing chlorophylls *a* and *b* and the xanthophylls, lutein, neoxanthin and zeaxanthin as major accessory pigments (Larkum *et al.* 2003). *Chlorella* sp. is an eukaryotic green alga, strains of which can be found growing freshwater/brackish water and seawater. It is often used in algal culture studies and is a common resident of sewage ponds and prawn farms. The halotolerant chlorophyte, *Dunaliella salina,* is the basis of the β-carotene algal biotechnology industry and accumulates glycerol and many lipid compounds (Borowitzka and Borowitzka 1988, Borowitzka 1992, 1999; Sosik and Mitchell 1994, Giordano and Beardall 2009). For comparison, the vascular plant *Pisum sativum* L. (common pea) which is a commonly used benchmark species for photosynthetic studies (White and Critchley 1999, Ralph and Gademann 2005) has been included in the study. The diatom *Phaeodactylum* sp. (Bacillariophyta) has chloroplasts containing chlorophyll (Chl) $a + c_1$ and c_2 and the xanthophylls diatoxanthin and fucoxanthin as accessory pigments (Larkum *et al.* 2003). Diatoms are important primary producers in marine and freshwater habitats and many produce substantial amounts of hydrocarbons and other oily compounds (Falkowski and Raven 2007). Amongst the cyanobacteria, *Spirulina platensis* shows the most promise because of its high productivity and very crucially its tolerance of conditions which exclude most grazers and competitors (Talling *et al.* 1973, Richmond and Zhou 1999, Grobbelaar 2007).

Modulated Chl fluorometry, using the PAM fluorescence technique, provides a means to make rapid and accurate measurements of key photosynthetic parameters (Ritchie 2008b). PAM technology has made photosynthetic light-response curves (P_G *vs*. E) very easy to obtain, compared to much more time-consuming routines required for such measurements using oxygen electrode, ^{14}C-fixation or infrared gas analyser (IRGA) methods. For example, a rapid light curve (RLC) (White and Critchley 1999, Ralph and Gademann 2005) on an alga or a plant such as pea (*Pisum sativum*) can be measured in about 2 min compared to the hours required using other methods. As pointed out by Ralph and Gademann (2005) RLCs are not always comparable to classical P_G *vs*. E curves and need to be validated by comparing PAM and oxygen electrode-derived results, as done here. PAM techniques can be used to measure the electron transport rate (ETR) in both algae and land plants using various fitting curves of which the waiting-in-line curve has been shown to give a very good fit for most photoautotrophs (Ritchie 2008b).

While PAM fluorescence can be used as a good proxy for photosynthetic performance under certain conditions, it needs always to be compared with some other standard method of measuring photosynthetic rate. Use of the oxygen electrode is a very suitable and well-established means of doing this (Walker 1990), since it can be used to measure both photosynthetic rate and respiratory rate. Respiratory rate is a vital parameter of any study of P_N of algae and cannot be measured by PAM fluorescence means.

In the present study photosynthesis and respiration were measured in representative algae used in algal ponds [*Dunaliella* (Chlorophyta), *Chlorella* (Chorophyta) and *Phaeodactylum* (Bacillariophyta)]. P_G was measured using routine oxygen electrode methods or PAM fluorometry. Respiration measurements were made using oxygen electrode methods on all three algae. In order to be able to estimate P_G and P_N over the course of a diel cycle, the effect of solar angle upon light transmitted across a pond surface was quantified using Snell's and Fresnel's equations (Lorrain *et al.* 1988). Integrative methods could then be employed to calculate total P_N for ponds in full sunlight and over the course of a day (Ritchie 2010). The primary emphasis in the study was to develop a better model for estimating photosynthesis in shallow ponds and how to establish the optimum production for a given depth rather than achieving the maximum production.

Materials and methods

Culture conditions and experimental materials: *Chlorella* sp. (Sydney University Algal Culture Collection) was grown in BG-11 medium (Allen 1973). The marine diatom, *Phaeodactylum* sp. (Sydney University Algal Collection) was cultured in enriched seawater C medium (MBIC medium N^o 8) (Ritchie 2006, Gloag *et al.* 2007, Ritchie 2008a,b). *Dunaliella salina* was a kind gift from Prof. Michael Borowitzka (Murdoch University, Western Australia) and it was grown in MBIC medium No 8 with the salinity boosted to 150‰ by addition of extra NaCl. All cultures were unialgal (not axenic) but did not have fungal contamination. The common pea plants (*Pisum sativum* L.) were grown in pots of standard potting mix in the same greenhouse as where the algae were grown. They were watered and fertilised regularly by the gardener.

Chlorella and *Phaeodactylum* were kept in a culture room on an orbital shaker (≈ 80 rpm) fitted with overhead fluorescent lights (*Sylvania Gro-Lux*, *Osram*, Münich, Germany) in continuous light at 27°C, which is near to optimal growth temperature for the three algae (*Phaeodactylum*: Fawley 1984, *Chlorella*: Dauta *et al.* 1990, *Dunaliella*: Sosik and Mitchell 1994). *Dunaliella salina* cells are very fragile: cultures were kept routinely as a static culture in the culture room with daily stirring. The light intensity in the culture room or on a south-facing window sill was approximately 100 μmol m^{-2} s^{-1} (PPFD 400–700 nm), using a *Li-Cor* photon flux meter *Model LI-189* (*Li-Cor*, Lincoln, NE, USA). *Chlorella, Phaeodactylum* and *Dunaliella* were acclimated to full sunlight in the air-conditioned greenhouse (≈ 25°C) in a two-stage process: cultures kept routinely under fluorescent lights in the culture room were gradually acclimated to natural sunlight in the greenhouse by covering with shade cloth for 3–4 d and gradually increasing illumination: full acclimation took about 2 weeks. Acclimated *Chlorella* and *Phaeodactylum* cultures in the greenhouse were aerated with air. *Dunaliella* cultures acclimated very easily to the greenhouse and were examined and stirred by hand daily. Cultures were grown in the greenhouse semicontinuously in 0.5-l conical flasks with about 300 ml of culture medium. About 1/3 of the volume was replaced by new culture medium every 2 d. Culture densities were maintained at approximately 2 g(Chl *a*) m^{-3} (Table 1).

Pulse amplitude modulation (PAM) fluorometry employs the fluorescence emission of Chl that results from a brief but strong light pulse of known intensity which induces multiple turnovers of photosystem II (PSII). The technique measures variable fluorescence in response to brief pulses of light and with this information one can estimate ETR (Ritchie 2008b). PAM fluorometers were primarily designed for use on vascular plants which have Chl *a* as the primary photosynthetic pigment and Chl *b* as the main auxiliary photosynthetic pigment but they can be used on most photosynthetic organisms with Chl *a* as their primary photosynthetic pigment. It is not well understood why it is difficult to obtain consistent and reliable results using PAM machines on most types of cyanobacteria (Ritchie 2008b).

Light-saturation curve measurements on *Chlorella*, *Phaeodactylum*, and pea leaves were made using a *Junior PAM* portable Chl fluorometer (*Gademann Instruments*, Wurzburg, Germany) fitted with a 1.5 mm diameter optic fibre and a blue diode light source. The Junior PAM uses a magnetic clamp to hold specimens about 1 mm from the end of the light pipe. PAM parameters (effective quantum yield, rETR, NPQ) were calculated using the *WIN-CONTROL* software (*2.133/03.00*) using standard settings for rapid light curves (*Heinz Walz*, Effeltrich, Germany) (Genty *et al*. 1989). The default absorptance factor of 0.84 and the default value of 0.5 for estimated absorption of light by PSI and PSII were used on the Junior PAM to calculate the relative electron transport rate (rETR) (*see* Appendix) (Ritchie 2008b). On the standard settings for a rapid light curve (White and Critchley 1999, Ralph and Gademann 2005), sets of PAM light curve measurements took about 88 s to complete with 10 s between actinic flashes of light and each flash of light was 0.8 s duration. The measuring light intensities were in order of increasing intensity.

Replicate samples of *Chlorella* and *Phaeodactylum* cells (usually 3- or 5-ml cell suspensions) were filtered onto *Whatman GF-C* glass fibre filters (*Whatman International*, Maidstone, England, UK) in a Millipore filtration apparatus for 25 mm filters (*Millipore*, Billerica, MA, USA), then dark treated in a Petri dish with disks of filter paper impregnated with seawater or BG-11 medium, as appropriate, for at least 10 min. Only one light-saturation experiment was run on each filter to avoid confounding effects of multiple experimental treatments. The inside diameter of the Millipore filtration apparatus was 15.9 mm and so the disks of algae adhering to the glass-fibre filter had a surface area of 198.6 × 10^{-6} m^2. The algal-impregnated disks provided highly reproducible material for experiments and a very favourable light geometry compared to using algal suspensions. Care was taken to avoid the algae-impregnated disks drying out. For Chl measurements of the pea leaves standard leaf disks were cut with a hole-punch (diameter = 6.3 mm, area = 31.17 × 10^{-6} m^2) and Chls in the leaf disks were extracted using the same protocol as that used for the algae.

Oxygen electrode studies: An oxygen electrode setup (*Hansatech*, England, UK) was used to measure respiratory rates of the three algae (Walker 1990). Oxygen solubilities in BG-11 medium (salinity equivalent to about 3‰) and for seawater (34.3‰) at 25°C were calculated using the algorithms of Garcia and Gordon (1992). To enable measurements of respiration and photosynthesis of *Dunaliella salina*, we developed an oxygen solubility algorithm to calculate O_2 solubility in hypersaline brines using the solubility data of Nishre and Ben-Yarkov (1990) for Dead Sea brine (280‰: O_2 sat = 45.3 mmol(O_2) m^{-3}), values for the solubility of oxygen calculated for pure water and normal seawater calculated using the algorithms of Garcia and Gordon (1992) and the data of Sherwood *et al*. (1991) for a variety of saline brines. The solubility of oxygen in 150‰ salinity at 25°C was estimated to be 102 mmol(O_2) m^{-3}. Respiration of twelve separate 1.0 ml samples of each algal culture was measured. Respiration of volumes of algal culture was calculated as mol(O_2) m^{-3} s^{-1}. For respiration measurements of the pea leaves standard leaf disks were cut with the 6.3 mm hole punch and placed in the O_2 electrode with BG-11 medium.

To prepare cells for O_2 electrode experiments, cells were centrifuged in a *Heraeus Labfuge 400R* (*Heraeus*,

Osterode, Germany). Low speeds were deliberately used: a speed of 1,500 rpm (428 × *g*) was used for *Chlorella* and *Phaeodactylum* but for *Dunaliella* a lower speed of 1,000 rpm (190 × *g*) was used. Cells were centrifuged for 15 min and the cells were carefully resuspended in fresh culture medium.

PAM measurements could not be performed on *Dunaliella* cells filtered onto glass fibre disks because the fragile cells were killed by the filtration process. P_G *vs*. E curves using the oxygen electrode were performed on statistically independent samples of *Dunaliella* cell suspension. A simple slide projector was used as the light source (*Leitz Pradovit 250, Leitz*, Wetzlar, Germany) using an *Osram Xenophot HLX 64655* lamp (*Osram*, Augsburg, Germany) and the projector was fitted with the standard 680 nm cut-off filter for infrared light. Irradiance through the *Hansatech* O_2 electrode apparatus with only water in the electrode chamber was measured using the *LiCor* quantum light meter. Balzers neutral density filters (*Balzers*, Fürnstentum, Liechenstein) were used to adjust the irradiance over the range 56 to 2,000 μmol m^{-2} s^{-1} PPFD. A sample of cells was placed on the apparatus and *R* measured over the course of about 10 min, the light source was then turned on and P_N was measured. The cell suspension was then removed and replaced by another sample of cell suspension for another determination of *R* and P_N. P_G was calculated as $P_N - R$ or $P_N + |(R)|$. P_G *vs*. E curves were fitted using the waiting-in-line model (Ritchie 2008b, Ritchie and Bunthawin 2010a,b; Ritchie 2012).

Chl determinations: After photosynthetic electron transport determinations, Chl was extracted from the glass fibre disks using ethanol (99.5% ethanol neutralised with $MgCO_3$) and Chls determined using the algorithms of Ritchie (2006, 2008a). Chl determinations of cell suspensions were made after centrifuging a known volume at 3,850 × *g* (4,500 rpm), discarding the supernatant and adding 3 ml of alcohol. It was difficult to extract Chl from *Chlorella* on glass fibres disks or as pellets in ethanol unless the cells were heated in alcohol in a water bath at about 80°C for about 3 min. After dissolving the Chls, the Chl extracts were removed from the glass fibre disks or pellets by transfer after centrifugation and the alcohol extracts made up to 3 ml and stored at –20°C as described previously (Ritchie 2006, 2008a). Extracts were stored in the dark in a freezer at –20°C before spectrophotometric assay for as short a time as practicable.

Chls were determined from spectrophotometric readings made using a *Shimadzu UV-2550* UV-visible spectrophotometer (*Shimadzu*, Kyoto, Japan) using quartz cuvettes as described previously (Ritchie 2006, 2008a). Generally Chl assays were made within a few hours of extraction or the next day. Heat-treated Chl extracts (chlorophyllase is readily deactivated by heating) appear to be stable and could be stored at –20°C indefinitely. Replicate disks from the same batch of cells generally varied by less than ± 2% in Chl content.

Calculation of rETR on a Chl basis: The *Walz* software calculates ETR on a surface area basis (the surface area of the object illuminated by the beam of light) as mol(e^-) m^{-2} s^{-1}: this can be converted to mol(O_2) m^{-2} s^{-1} assuming $4e^-/O_2$. For *Chlorella* and *Phaeodactylum* the diameter of the glass fibre disks of algae or leaf disks and their Chl content were both known and so mg of Chl per square metre could be calculated. rETR (refer to Appendix) in mol(O_2) m^{-2} s^{-1} was converted to mol(O_2) mg^{-1}(Chl *a*) h^{-1} using the Chl assays [as mg(Chl *a*) m^{-2}]. Since the Chl *a* content per unit volume of the cultures was also known, photosynthesis and respiration could also be expressed as per unit volume of culture [mol(O_2) m^{-3} s^{-1}] and for a water column of known depth they could also be expressed on a pond surface area basis [mol(O_2) m^{-2} s^{-1}] or with caution on a daily or even annual basis.

Curve fitting: The waiting-in-line model was used to fit P_G *vs*. E curves using the form of the equation that is easiest to fit using nonlinear least squares methods (Ritchie and Bunthawin 2010a,b; Ritchie 2012 and *see* Appendix, Eq. 3). Fitting of the waiting-in-line equation gave estimates of the optimum irradiance (E_{opt}) and the maximum rETR ($rETR_{max}$) or P_{Gmax} where the waiting-in-line equation was used to fit oxygen electrode-based estimates of gross photosynthesis.

Statistics: All errors quoted are ± 95% confidence limits. The number of data points is quoted in brackets (*n*). Curves were fitted by nonlinear least squares fitting and the asymptotic errors calculated by matrix inversion (Johnson and Faunt 1992, Ritchie 2006, 2008a,b; Gloag *et al*. 2007). The *EXCEL* ® routines for calculating the oxygen solubility in hypersaline waters, the curve fitting routines for P_G *vs*. E curves and the reflectance/ refraction calculator are available upon request.

Results

Gross photosynthesis as related to depth in shallow ponds: Our approach was to grow algae under controlled conditions and then to measure photosynthesis under defined conditions using a PAM fluorometer and to measure respiration in the dark using an oxygen electrode technique. Fig. 1 shows a plot of P_G (estimated as rETR/4) against irradiance for *Chlorella,* which had been grown under natural light in a greenhouse for several weeks. For comparison, we also measured common garden pea leaves (Table 1), also grown under natural

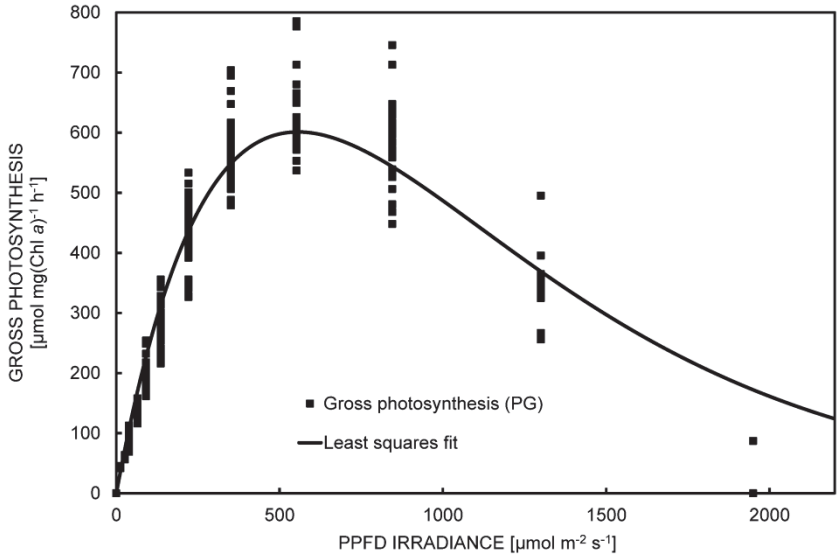

Fig. 1. Light saturation data on *Chlorella* grown in a greenhouse in full sunlight fitted to the waiting-in-line model. Gross photosynthesis (P_G) has been estimated as rETR/4 and have been standardised on the Chl *a* content of cell suspensions impregnating glass fibre disks. Irradiance (E) is in 400–700 nm PPFD. Five ml samples of cell suspensions were filtered onto glass fibre disks. The parameters for the fitted curve are shown in Table 1.

Table 1. Gross photosynthesis *vs*. irradiance fitted to waiting-in-line model. E_{opt} – optimum irradiance; α_0 – asymptotic maximum photosynthetic efficiency; P_{Gmax} – maximum gross photosynthesis. * PAM not used, but O_2 electrode methods.

Parameter	*Chlorella* (Culture room)	*Chlorella* (Glasshouse)	*Dunaliella* (Glasshouse)	*Phaeodactylum* (Culture room)	*Phaeodactylum* (Glasshouse)	*Pisum sativum* (Glasshouse)
E_{opt} [µmol m^{-2} s^{-1} PPFD]	238 ± 8.05	556 ± 15.5	968 ± 90.44	339 ± 10.9	316.6 ± 6.76	730 ± 32.4
α_0 [10^{-9} g(Chl *a*)$^{-1}$ m^2]	777 ± 31.0	816.1 ± 26.6	196 ± 28.2	451 ± 17.3	1057 ± 26.5	162 ± 8.42
P_{Gmax} [µmol mg(Chl *a*)$^{-1}$ h^{-1}]	243 ± 5.26	601.2 ± 10.05	251.1 ± 14.33	202.3 ± 4.24	443 ± 5.86	157 ± 4.20
Correlation (*r*),	*r* = 0.9881,	*r* = 0.9812,	*r* = 0.8535,	*r* = 0.9772,	*r* = 0.9599,	*r* = 0.9704,
Data points (*n*)	*n* = 324	*n* = 342	*n* = 72	*n* = 324	*n* = 378	*n* = 324
Chlorophyll ratios (*n*)	*b*/*a* = 0.529 ± 0.0053 (12)	*b*/*a* = 0.457 ± 0.00358 (12)	*b*/*a* = 0.418 ± 0.0014 (12)	c_1c_2/*a* = 0.263 ± 0.0030 (12)	c_1c_2/*a* = 0.246 ± 0.00401 (12)	*b*/*a* = 0.548 ± 0.00614 (12)
[mg(Chl *a*) m^{-2}](*n*)	49.4 ± 1.67 (12) (GF disk)	49.6 ± 1.59 (12) (GF disk)	*	60.5 ± 1.24 (12) (GF disk)	32.0 ± 1.135 (12) (GF disk)	277 ± 22.6 (12) (leaf disks)
[g(Chl *a*) m^{-3}] of culture (*n*)	3.26 ± 0.110 (12)	1.97 ± 0.0629 (12)	2.74 ± 0.0231 (12)	2.40 ± 0.0492 (12)	2.11 ± 0.0747 (12)	Not applicable

light in the greenhouse; with these photoinhibition was much less severe at high irradiance than for *Chlorella*. The pea leaves acted as a benchmark material and the results are similar to those found previously for peas (White and Critchley 1999), subterranean clover grown in a field (Ritchie 2008b), orchids, pineapples and water lilies growing in tropical sunlight (Ritchie and Bunthawin 2010a,b; Ritchie 2012). The *Chlorella* and the pea leaf data sets could be fitted to the waiting-in-line equation (Eq. 5) without log/log transformation of the data. The statistics for the fitted curves are shown in Table 1 for *Chlorella,* the common garden pea *(Pisum sativum)*, *Dunaliella* and *Phaeodactylum*. Preliminary work showed that *Chlorella* gave quite different results if the test cultures were not fully acclimated to growing in full sunlight. In the case of *Chlorella* we found that acclimation was slow and took 7–10 d. Problems arising from the time needed for photoacclimation by algae are often overlooked (MacIntyre *et al.* 2002). Photosynthetic efficiency (α_0) calculated on a surface area basis is a dimensionless number because all the units cancel. However, in the present study α_0 was standardised on a Chl basis and so had the dimensions m^2 g^{-1}(Chl *a*) (Table 1). *Chlorella* acclimated to growing in the greenhouse had the highest saturating photosynthetic rate on a Chl basis (P_{Gmax} = 601 ± 10 µmol(O_2) mg^{-1}(Chl *a*) h^{-1}) and garden peas had the lowest (P_{Gmax} = 157 ± 4.20 µmol(O_2) mg^{-1}(Chl *a*) h^{-1}). The saturating light intensity was only 317 ± 6.8 µmol m^{-2} s^{-1} PPFD for *Phaeodactylum,* 556 ± 16 µmol m^{-2} s^{-1} PPFD for *Chlorella*, higher for garden peas (730 ± 32 µmol m^{-2} s^{-1} PPFD) and the highest for *Dunaliella* (968 ± 90.4 µmol m^{-2} s^{-1} PPFD). The pea leaves had a high saturating light intensity and showed about 50% photoinhibition in full sunlight. This is a typical value for a vascular plant that is a "sun plant", hence its use as a benchmark species in the present study (Ritchie 2010, 2012;

Table 2. Photosynthetic parameters for a pond in direct full sunlight (2,207 μmol m^{-2} s^{-1}) PPFD (allowing for 2% reflection). k_L – light attenuation constant; E_{opt} – optimum irradiance; P_N – net photosynthesis; P_{Gmax} – maximum gross photosynthesis.

Parameter	*Chlorella* (Glasshouse)	*Dunaliella* (Glasshouse)	*Phaeodactylum* (Glasshouse)
Light attenuation (k_L) [m^{-1}, *n*]	59.0 ± 1.20 (20)	60.8 ± 0.972 (20)	82.2 ± 0.946 (20)
Respiration [μmol mg(Chl *a*)$^{-1}$ h^{-1}]	–22.2 ± 3.34 (12)	–21.1 ± 2.08 (72)	–39.3 ± 4.94 (12)
Respiration (volume basis)	–20.1 ± 3.04 (12)	–16.1 ± 1.59 (72)	–23.1 ± 2.90 (12)
[μmol m^{-3} s^{-1}]	[1.97 g(Chl *a*) m^{-3}]	[2.74 g(Chl *a*) m^{-3}]	[2.11 g(Chl *a*) m^{-3}]
Depth of maximum P_N in the pond [mm]	20.2 ± 0.616	14.0 ± 1.30	23.6 ± 0.534
for given Chl *a* density	[1.97 g(Chl *a*) m^{-3}]	[2.74 g(Chl *a*) m^{-3}]	[2.11 g(Chl *a*) m^{-3}]
P_{Gmax} [μmol mg(Chl *a*)$^{-1}$ s^{-1}]	597 ± 9.83	258 ± 16.2	404 ± 6.32
P_{Gmax} [μmol m^{-3} s^{-1}]	326 ± 11.8	197 ± 12.5	237 ± 9.14
Correlation (*r*), data points (*n*)	*r* = 0.8877, *n* = 288	*r* = 0.8498, *n* = 72	*r* = 0.8760, *n* = 336
Integration of pond P_N			
Compensation depths [mm]	No upper point 730 (lower)	No upper point 488 (lower)	7.1 (upper) 339 (lower)
Optimum depth [mm] for maximum total P_N	87	70.8	63
for given Chl *a* density	[1.97 g(Chl *a*) m^{-3}]	[2.74 g(Chl *a*) m^{-3}]	[2.11 g(Chl *a*) m^{-3}]
Maximum total P_N (μmol m^{-2} s^{-1}]	12.6 ± 0.758	6.45 ± 0.408	6.08 ± 0.350
Respiration in dark [μmol m^{-2} s^{-1}]	–1.75 ± 0.264	–1.14 ± 0.113	–1.45 ± 0.183

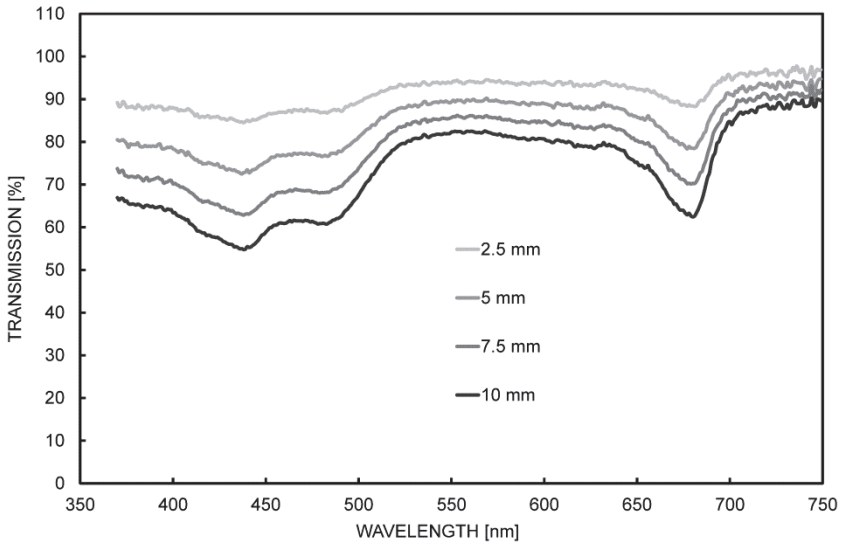

Fig. 2. Transmission of light through cultures of *Chlorella vs*. wavelength of light measured using a Taylor-sphere from 370 to 750 nm. The cells were grown under fluorescent lights in a culture room. The blue absorption peak was at 438 nm and the red peak at 679 nm.

Ritchie and Bunthawin 2010a, b). *Dunaliella* and *Chlorella*, grown under high-light conditions, would also qualify as "sun plants". *Dunaliella* cultures in the culture room were green but bright orange in the glasshouse. Comparisons of the *Chlorella* and *Phaeodactylum* cultures grown in the culture room compared to the glasshouse showed that *Chlorella* adjusted its optimum irradiance (E_{opt}) in the glasshouse but *Phaeodactylum* did not (Table 1). All statistical fits to the waiting-in-line model were highly significant ($p << 0.001$).

Table 1 presents the data on the Chl contents of the algal suspensions used for the present study expressed as Chl *a* per unit volume of culture and Chl *a* content of the pea leaves expressed on a leaf surface area basis. The Chl *b*/*a* ratio of *Chlorella* cells grown in the greenhouse was considerably lower than for those grown in the culture room whereas the Chl c_1c_2/*a* ratio of *Phaeodactylum* did not differ greatly. The Chl ratios found in the present study for *Chlorella* and *Phaeodactylum* grown in the culture room were similar to those found for the algae in previous studies (Ritchie 2006, 2008a).

Net photosynthesis as related to depth in shallow ponds: Table 2 presents the data obtained for modelling the P_N of the microalgae as a function of depth in shallow ponds obtained for cultures that were grown in the greenhouse. Table 1 clearly shows that culture-room grown material would not be appropriate to use for such modelling. Transmission of light through algal suspensions was measured using an Integrating-Taylor-Sphere (*ISR-240A*, *Shimadzu*, Kyoto, Japan) and modelled using Eq. 4 and the irradiance constants calculated for the blue-peak absorption. Fig. 2 shows attenuation *vs*. light path for *Chlorella* acclimated to the greenhouse. A cell suspension

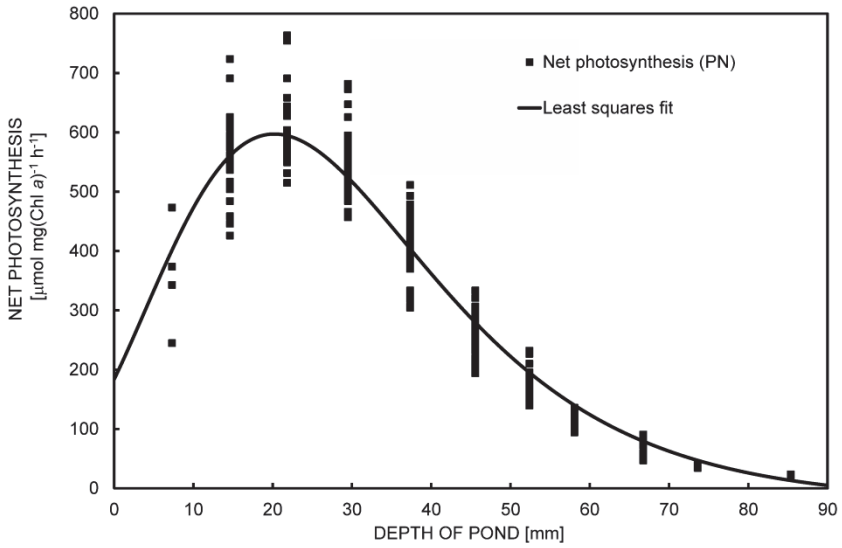

Fig. 3. Net photosynthesis (P_N) of a *Chlorella* culture grown in full sunlight *vs*. depth [mm] calculated using Eq. 6 (Appendix). Photosynthesis information was derived from the same data set used to plot the gross photosynthesis *vs*. irradiance curve shown in Fig. 1 and respiratory data and the light attenuation data from Table 2.

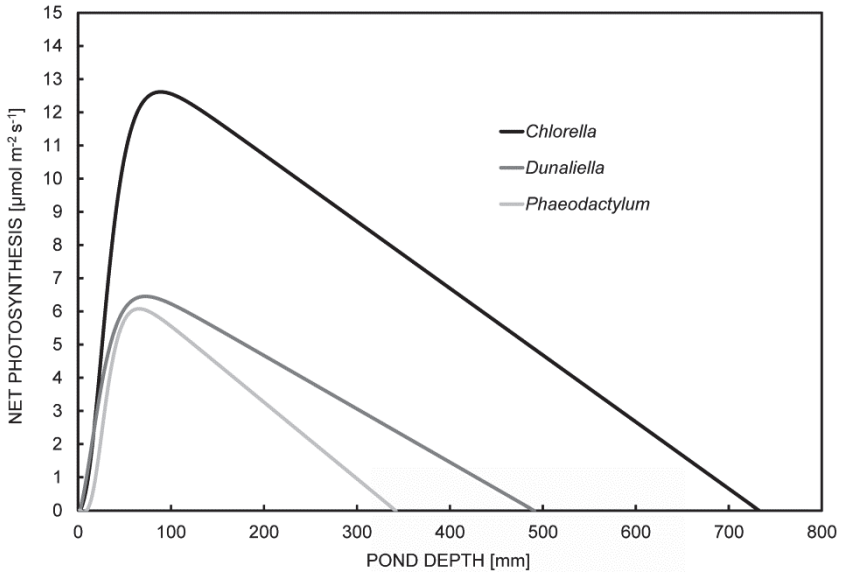

Fig. 4. Integrated total net photosynthesis (P_N) of a greenhouse-grown *Chlorella, Dunaliella* and *Phaeodactylum* cultures *vs*. depth calculated using Eq. 8 (Appendix) calculated using the data in Tables 1 and 2 for the mean Chl *a* densities in the cell suspensions [g(Chl *a*) m^{-3}]. P_N was calculated on the basis of the surface area of the pond and the sun was taken to be directly overhead and 2% of the light is reflected off the surface of the pond.

only 10 mm thick absorbed about 55% of incident light at 438 nm and had an attenuation constant (k_L) of 59.00 ± 1.2 m^{-1} (n = 20). For *Dunaliella,* k_L = 60.82 ± 0.972 m^{-1} (n = 20) at 438 nm and for *Phaeodactylum,* k_L = 82.2 ± 0.95 m^{-1} (n = 20) at 437 nm. The Chl contents per unit volume for the cultures used are shown in Table 1.

Fig. 3 shows P_N of a *Chlorella* culture grown in the greenhouse [µmol(O_2) mg^{-1}(Chl *a*) h^{-1}] *vs*. depth [mm] calculated using Eq. 6. Photosynthetic information was derived from the same data set used to plot the gross photosynthesis *vs*. irradiance curve shown in Fig. 1 and respiratory data and the light attenuation data from Table 2. At high irradiances, near the surface of the algal suspension, there was strong photoinhibition. Net photosynthesis was strongly inhibited (but still positive), maximum photosynthesis was reached at a depth of about 20 mm, after which P_N fell to zero at about 90 mm. At that depth, the ambient light would be only about 7 µmol m^{-2} s^{-1} PPFD.

Fig. 4 shows P_N of the algal suspension integrated-over-depth (Eq. 8) using the data in Tables 1 and 2. P_N rates in Fig. 4 are expressed as mol m^{-2} s^{-1} based upon the mean values for the Chl *a* per unit volume of culture shown in the bottom line of Table 1 to calculate P_N per unit surface area of the pond for a given depth of the pond. Net photosynthesis was calculated for a pond with full sunlight directly overhead (≈ 2,200 µmol m^{-2} s^{-1} PPFD) but allowing for 2% reflection off the water surface (Snell's Law for freshwater, seawater and 150‰ hypersaline brine). In the case of *Chlorella,* increasing the depth of the pond from zero depth increased the total P_N of the water column; the optimum depth for a pond with this particular batch of *Chlorella* culture was 87 mm (as would be expected from Fig. 3). Respiration by pond layers below the optimum depth of 87 mm progressively overwhelmed photosynthesis in the photic zone until a pond with a depth of only 730 mm would have a zero P_N. The shape of the overall curve is very similar to that

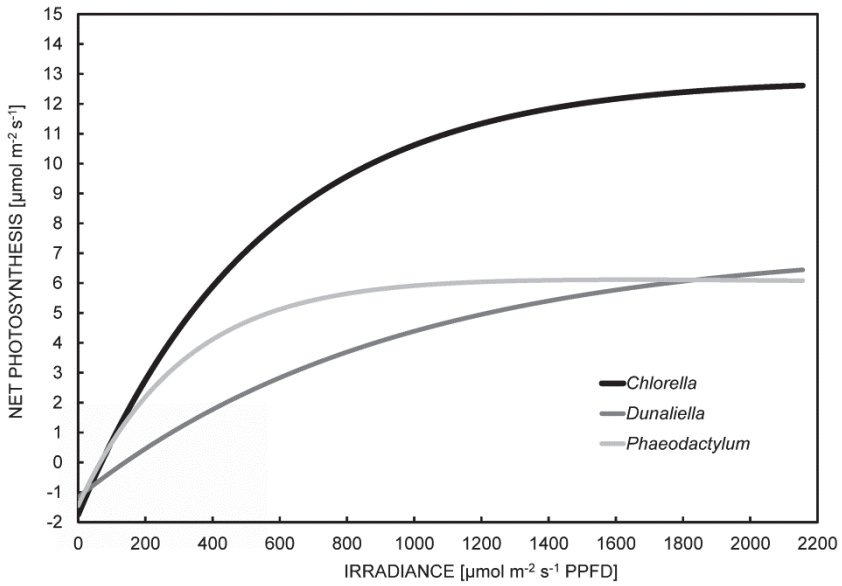

Fig. 5. Integrated total net photosynthesis (P_N) of a greenhouse-grown *Chlorella, Dunaliella*, and *Phaeodactylum* pond cultures on a pond surface area basis *vs*. irradiance calculated using Eq. 8 (Appendix) but holding the pond depth at the optimal depth (Table 2: *Chlorella*, 87 mm; *Dunaliella* 70.8 mm; *Phaeodactylum* 63 mm). The sun was taken to be directly overhead and 2% of the light is reflected off the surface of the pond. Such a pond failed to reach its light compensation point until direct incident irradiance exceeds a minimum irradiance (*Chlorella*, 69.5 µmol m^{-2} s^{-1} PPFD; *Dunaliella*, 139.5 µmol m^{-2} s^{-1} PPFD; *Phaeodactylum*, 65.4 µmol m^{-2} s^{-1} PPFD).

calculated by Grobbelaar *et al.* (1990) and Sukenik *et al.* (1991) even though they used different models for their P_G *vs*. irradiance curves. The *Dunaliella* cultures in the glasshouse behaved similarly to *Chlorella* but there was less surface inhibition (due to its higher E_{opt}) but it had lower maximum photosynthesis. The optimum depth of the water column was 71 mm. Net photosynthesis of *Dunaliella* fell off to zero at 488 mm. *Phaeodactylum* was much less able to cope with growth in full sunlight than *Chlorella* or *Dunaliella* (Tables 1, 2; Fig. 4). Photoinhibition near the surface was so severe that water columns of these algae exhibited two compensation points where total P_N was less than zero: the upper compensation depth was 7.1 mm (Table 2). The maximum P_N for *Phaeodactylum* was at 63 mm mainly as a consequence of *Phaeodactylum* having the highest respiratory rate (Table 2). The total P_N of a water column of the alga *vs*. depth (Eq. 8) fell rapidly to zero at a depth of only 339 mm.

Fig. 5 models the effects of increasing E upon the total P_N integrated for the water column of a *Chlorella* pond with an optimalised depth of 87 mm, a *Dunaliella* pond with an optimalised depth of 71 mm and a *Phaeodactylum* pond with a fixed depth of 64 mm. P_N integrated over the full depth of the ponds *vs*. E was calculated using Eq. 8. As in the case for Fig. 4, about 2% of the incident irradiance would have been reflected off the water surface. The curves have a slowly saturating shape, with an origin below zero. Light curves integrated over depth (Eqs. 8, 12) did not exhibit any apparent photoinhibition, even in full sunlight. Note that in Fig. 5 the photosynthetic rates are based on pond surface area (not a Chl basis). P_N of ponds with optimised depth in full sunlight was much poorer for *Dunaliella* and *Phaeodactylum* than for *Chlorella* (Table 2: *Chlorella*, 12.6 ± 0.758; *Dunaliella*, 6.45 ± 0.408; *Phaeodactylum*, 6.08 ± 0.350 µmol(O_2) m^{-2} s^{-1}). P_N was below zero for low E (compensation irradiance: *Chlorella*, 69.5 µmol m^{-2} s^{-1} PPFD; *Dunaliella*, 139.5 µmol m^{-2} s^{-1} PPFD; *Phaeodactylum*, 65.4 µmol m^{-2} s^{-1} PPFD).

Optimising net photosynthesis in shallow ponds: The implications for optimising the productivity of algal ponds can now be addressed, focusing on *Chlorella* because it gave the highest overall productivity. For optimum productivity from a culture of *Chlorella* cultures acclimated to full sunlight with Chl *a* content of about 2 g(Chl *a*) m^{-3} (Table 1), the optimal depth for the pond was only 87 mm. Shallower cultures did not harvest all the available light efficiently (and it would encourage unwanted growth of other algae on the bottom of the pond) and having a pond deeper than 87 mm led to lower integrated P_N due to respiratory losses by the cells in the deeper layers below the photic zone. The maximum total P_N was 12.6 ± 0.758 µmol(O_2) m^{-2} s^{-1} on a surface area basis for a pond 87 mm deep or on a volume basis 1.10 ± 0.0675 µmol(O_2) m^{-3} s^{-1}. Optimum net productivity expressed on the basis of the surface area of pond on a carbon basis would be 0.152 ± 0.00911 g(C) m^{-2} s^{-1} or 13.19 ± 0.811 mg(C) m^{-3} s^{-1} in normal incident full sunlight (Figs. 4, 5), assuming a O_2/C ratio of 1 in photosynthesis (Westlake 1963). This O_2/C ratio neglects photorespiration and so overestimates actual C-fixation by the Calvin-Benson cycle (Falkowski *et al.* 1994, Larkum *et al.* 2003, Falkowski and Raven 2007). Respiration by the 87 mm deep water column of algal suspension considerably reduced the net daily P_N of the pond on a surface area basis (R = –1.75 ± 0.264 µmol(O_2) m^{-2} s^{-1} or 21.0 ± 3.17 µg(C) m^{-2} s^{-1}).

It is possible to calculate daily P_N if information on the time course of daily solar irradiance and solar angles are available and the reflection and refraction properties of the pond surface are also taken into account (*see* Appendix). The equation describing photosynthesis of

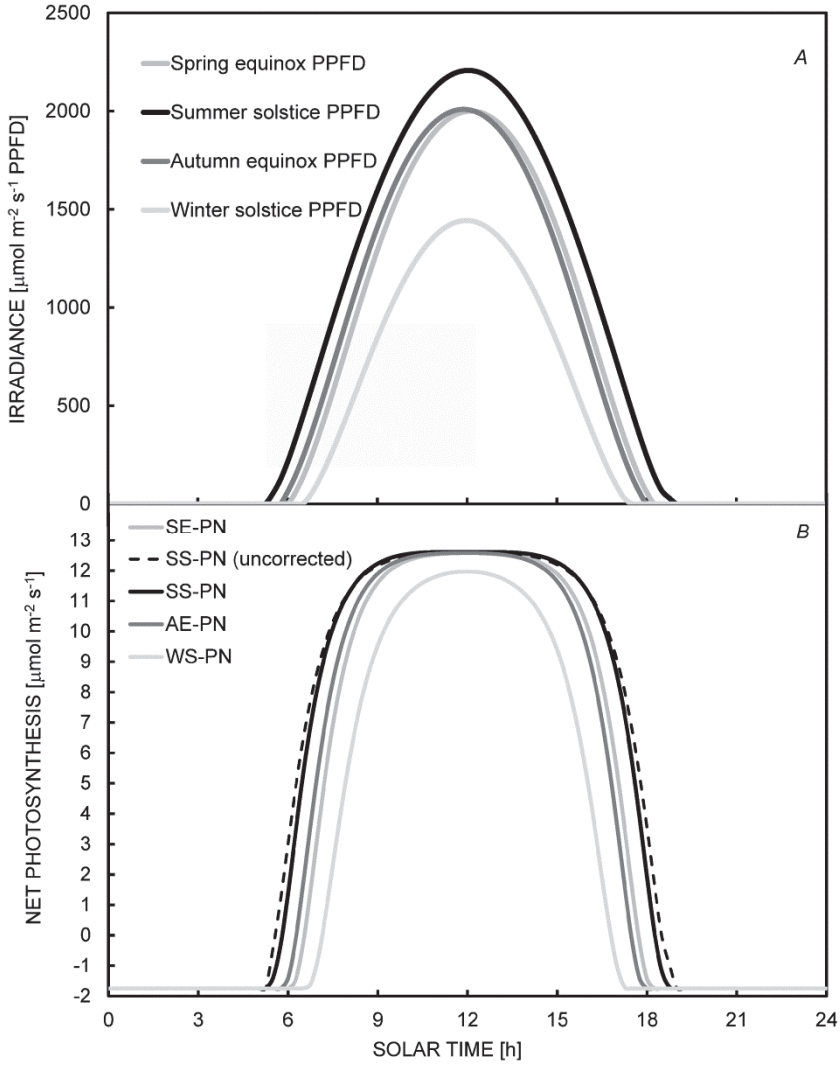

Fig. 6. Estimating the net photosynthesis (P_N) of a pond. *A*: The PPFD irradiance *vs*. time of day on a pond located at the tropic of Cancer for the spring equinox (SE), summer solstice (SS), autumn equinox (AE) and winter solstice (WS). *B*: The estimated P_N of a *Chlorella* pond 87 mm deep *vs*. time of day calculated using Eq. 12 (Appendix), taking reflection and refraction effects into account. During spring, summer and autumn P_N is approximately proportional to irradiance over the course of the day (PN-SE, PN-SS, PN-AE and PN-WS). For comparison, the time course for photosynthesis in midsummer making no allowances for reflection, refraction and the light path taken by transmitted irradiance in the pond is also shown (Appendix, Eq. 8). Neglecting reflection, refraction and the lengthening of the light path in the pond at low solar angles overestimates P_N in the morning and afternoon [compare PN-SE (uncorrected) with the correctly calculated estimate, PN-SE].

a pond with the sun directly overhead (Eq. 8) can be modified to allow for reflection and refraction and changes in the light path due to the angle of the rays of the sun in the pondwater (Eq. 12). Tables of light transmission, refraction and relative light path *vs*. solar angle for freshwater, seawater and *Dunaliella* brine at 25ºC are provided as supplementary material.

As a worked example we estimated total P_N for ponds at the tropic of Cancer for the spring equinox, summer solstice, autumn equinox and winter solstice. The *Smarts* software (*SMARTS 2009*, Ritchie 2010) was used to calculate solar angle and PPFD at 15-min intervals over the course of the day. Eqs. 9, 10, and 11 were used to calculate the angle of refraction in the water of the pond, the relative light path and the proportion of transmitted light for a given solar elevation.

To illustrate how the transmitted irradiance was calculated, consider a *Chlorella* pond with a depth of 87 mm in the early morning when the solar angle is 15º. The incident PPFD would be about 434 μmol m^{-2} s^{-1}. The angle to normal of the transmitted light would be 46.46º, the relative light path factor (x_{rel}) would be 1.452 and the proportion of transmitted light (E_{trans}) would be 0.788. Hence, 21.2% of incident light would simply bounce off the water surface. The actual light path inside the pond would be 1.452 × 87 = 126 mm and the transmitted irradiance penetrating the pond (E_t) would be 434 × 0.788 = 342 μmol m^{-2} s^{-1} PPFD.

Fig. 6*A* shows incident irradiance on a pond surface at the tropic of Cancer for the spring equinox, summer solstice, autumn equinox and winter solstice. The summer maximum with the sun directly overhead is ≈ 2,200 μmol m^{-2} s^{-1} PPFD for a daylength of about 13.75 h. The spring and autumn equinoxes provide a midday maximum of ≈ 2,000 μmol m^{-2} s^{-1} PPFD and about 12 h of sunlight. The incident irradiance at the winter solstice is ≈ 1,442 μmol m^{-2} s^{-1} PPFD and about 10.5 h of sunlight. Calculated P_N for the different seasons for a *Chlorella* pond are shown in Fig. 6*B*. During spring, summer, and autumn P_N is approximately proportional to irradiance over the course of the day. During winter P_N is reduced compared to the warmer months but it is notable that

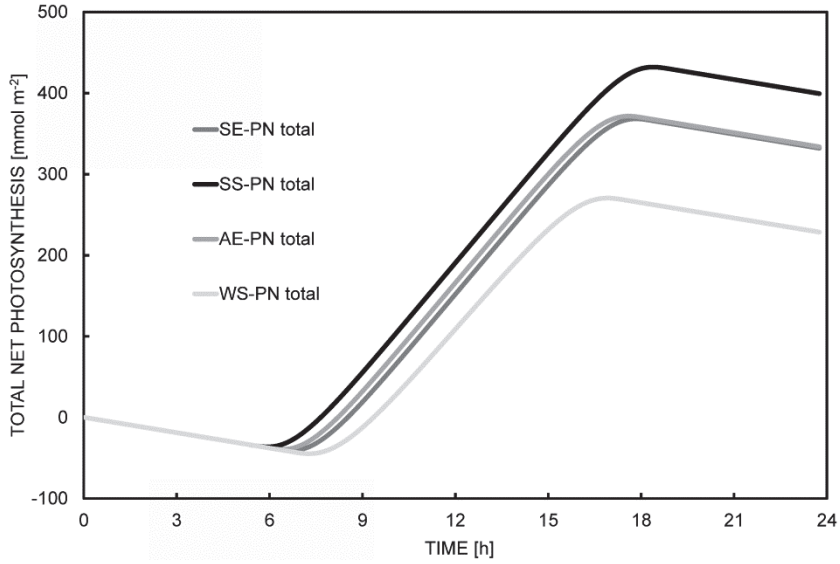

Fig. 7. In Fig. 6*B* net photosynthesis (P_N) was calculated at 15-min intervals and so the time course of daily P_N of the *Chlorella* pond can be calculated by summing the calculated rates over each time interval. The overall daily P_N (midnight to midnight) was; spring equinox (PN-SE), 330 ± 30 mmol(O_2) m^{-2}, summer solstice (PN-SS), 398 ± 32 mmol(O_2) m^{-2}, autumn equinox (PN-AE), 332 ± 30 mmol(O_2) m^{-2} and winter solstice (PN-WS) 227 ± 28 mmol(O_2) m^{-2}.

Table 3. Estimated daily photosynthesis and respiration of *Chlorella*, *Dunaliella* and *Phaeodactylum* ponds at the tropic of Cancer with set optimum depths. P_G − gross photosynthesis expressed on an oxygen basis; P_N – net photosynthesis; R – respiration.

	[mmol m^{-2} d^{-1}]			[g(C) m^{-2} d^{-1}]		
Chlorella	P_G	P_N	R	P_G	P_N	R
Spring equinox	482 ± 19.9	330 ± 30.3	151 ± 22.8	5.78 ± 0.239	3.96 ± 0.364	1.82 ± 0.274
Summer solstice	549 ± 22.7	398 ± 32.2	151 ± 22.8	6.59 ± 0.273	4.77 ± 0.387	1.82 ± 0.274
Autumn equinox	483 ± 20.0	332 ± 30.4	151 ± 22.8	5.80 ± 0.240	3.99 ± 0.364	1.82 ± 0.274
Winter solstice	378 ± 15.6	227 ± 27.7	151 ± 22.8	4.54 ± 0.188	2.72 ± 0.332	1.82 ± 0.274
Dunaliella	P_G	P_N	R	P_G	P_N	R
Spring equinox	229 ± 15.0	131 ± 17.9	98.4 ± 9.73	2.75 ± 0.180	1.57 ± 0.214	1.18 ± 0.117
Summer solstice	266 ± 17.4	168 ± 19.9	98.4 ± 9.73	3.19 ± 0.209	2.01 ± 0.239	1.18 ± 0.117
Autumn equinox	230 ± 15.1	132 ± 17.9	98.4 ± 9.73	2.76 ± 0.181	1.58 ± 0.215	1.18 ± 0.117
Winter solstice	169 ± 11.0	70.4 ± 14.7	98.4 ± 9.73	2.03 ± 0.132	0.846 ± 0.177	1.18 ± 0.117
Phaeodactylum	P_G	P_N	R	P_G	P_N	R
Spring equinox	279 ± 11.2	153 ± 19.4	126 ± 15.8	3.42 ± 0.135	1.84 ± 0.232	1.51 ± 0.189
Summer solstice	314 ± 12.7	189 ± 20.2	126 ± 15.8	3.77 ± 0.152	2.26 ± 0.243	1.51 ± 0.189
Autumn equinox	279 ± 11.3	154 ± 19.4	126 ± 15.8	3.35 ± 0.135	1.85 ± 0.233	1.51 ± 0.189
Winter solstice	231 ± 9.31	106 ± 18.3	126 ± 15.8	2.77 ± 0.112	1.27 ± 0.220	1.51 ± 0.189

lower maximum irradiance results in lower photoinhibition and so the shape of the daily course of photosynthesis is substantially different in winter compared to that found in spring, summer and autumn. For comparison, the time course for photosynthesis in midsummer making no allowances for reflection, refraction and the light path taken by transmitted irradiance in the pond is also shown (Eq. 8). As would be expected, neglecting reflection, refraction and the lengthening of the light path in the pond does not affect the estimate of P_N at midday but overestimates P_N in the morning and afternoon (overall by ≈ +4%). Compare the case shown in Fig. 6*B* where P_N for the summer solstice has been calculated: with and without the necessary corrections [SS-PN *vs*. SS-PN (uncorrected)].

Irradiance was calculated at 15-min intervals and so total daily P_N can be estimated by summation from midnight to midnight. At night there was a linear respiratory rate. During daylight P_G and R occured. After sunrise photosynthesis gradually increased and during most of the daylight hours there was a linear net gain of photosynthesis with time, before levelling off in the late afternoon and then there was linear dark respiration until midnight. For *Chlorella* (Fig. 7), the overall daily P_N was: spring equinox, 330 ± 30.3 mmol(O_2) m^{-2} [3.964 ± 0.364 g(C) m^{-2}], summer solstice, 398 ± 32.2 mmol(O_2) m^{-2} [4.773 ± 0.387 g(C) m^{-2}], autumn equinox, 332 ± 30.4 mmol(O_2) m^{-2} [3.986 ± 0.364 g(C) m^{-2}] and winter solstice 227 ± 27.7 mmol(O_2) m^{-2} [2.713 ± 0.332 g(C) m^{-2}] (Table 3). Using the same procedures outlined above for *Chlorella*, the data shown in Tables 1 and 2 and the refractive indices of seawater and *Dunaliella* brine

(150‰) were used to estimate daily P_N by *Phaeodactylum* and *Dunaliella* ponds located at the tropic of Cancer with their depths adjusted to yield optimum P_N in the midday sun at the summer solstice (Table 3). Daily P_N in the *Dunaliella* and *Phaeodactylum* ponds were about half that of the *Chlorella* pond in spring to autumn and considerably lower than that found in *Chlorella* in winter because of the lower photosynthetic rates of these two algae and their lower P_G/R ratio.

Discussion

The calculations in this study of P_N of shallow ponds are based on real data from three species of microalgae, *Chlorella* sp., *Dunaliella salina* and *Phaeodactylum* sp., grown under realistic conditions in culture and measured by standard means. The results closely matched experimental results on *Isochrysis* grown in an actual pond setup (Sukenik *et al.* 1991). The results indicated clearly that optimal ponds should be very shallow with negligible algal biomass below the compensation depth and the algal density needs to be high to absorb as much useful irradiance as possible in the photic zone. Here we used parameters derived from real algae and we modelled net and gross photosynthesis. Using solar flux data over a diel cycle and refractive index values we derived a model by which underwater irradiance can be calculated at any depth for any algal concentration and for any latitude and time of day. From these data the efficiency of solar energy conversion can be estimated for shallow ponds at any position on the Earth's surface and for any season of the year. We used ponds located at the tropic of Cancer as worked examples. The productivity calculation is more complex than for vascular plant crops (Ritchie 2010) because refraction at the water surface alters the angle of transmission of the incident light and hence the length of the light path within the pond and some light simply bounces off the pond surface, particularly at low solar angles. In terrestrial plants the leaf area index is usually substantially greater than unity and leaves are presented at many different angles to sunlight and so at any one time only some leaves reflect large amounts of light. Floating water lily leaves are an exception to these generalisations (Ritchie 2012). Leaves are thin compared to the depth of a pond and so refraction and changes in the light path within leaves does not have the large effects found in a pond, and which are discussed below.

We employed the theory of waiting-in-line, to accurately fit the P_G *vs*. E curve at low, medium and high irradiance (Ritchie 2010). The equation has only two unknowns and it is a very good fit to most P_G *vs*. E curves except in the case of species which exhibit little or no photoinhibition at high irradiances, in which case the Eilers-Peeters (E-P) formulation might be more useful (Eilers and Peeters 1988, Duarte 2006). Unfortunately, the Eilers-Peeters equation has three unknowns and although featured in Walz software for their PAM machines it is more difficult to fit and the asymptotic errors of the fitted parameters can be very large. The waiting-in-line model was first introduced for modulation fluorometry light saturation curves by Gloag *et al.* (2007) and Ritchie (2008b) and can be derived from the behaviour of effective photosynthetic quantum yield *vs*. irradiance curves (Ritchie 2008b). Furthermore, the waiting-in-line model can be further developed to describe photosynthesis *vs*. depth of an algal suspension in a column of water with the sun directly overhead (Eq. 8, Engqvist and Sjöberg 1980, McBride 1992). Other fitting models with asymptotic maxima such as simple saturating exponential curves and Tanh are discussed by Ritchie (2008b). Snell's Law (Eq. 9) can be used to estimate the transmission angle of incident light and the relative light path can be calculated (Eq. 10). The Fresnel equation can then be used to calculate the proportion of incident light actually transmitted into the water column. Integration methods as discussed below can be used to predict the photosynthesis of a body of water over 24 h (Ritchie 2010).

We showed that photoinhibition at high irradiances was a major factor in limiting primary productivity in ponds because it affects Eq. 8 and hence also Eq. 12. If an alga has a low optimal irradiance (E_{opt}) the curve is strongly rectilinear (*Phaeodactylum*, Fig. 5) whereas if an alga can tolerate high irradiances (E_{opt} is high) the curve is more linear and so photosynthesis is more directly proportional to irradiance (as shown in the theoretical models of Engqvist and Sjöberg 1980 and McBride 1992). This has not been given sufficient attention in many previous analyses of primary production in algal ponds (Kroon *et al.* 1989, Richmond and Zou 1999, MacIntyre *et al.* 2002), where photoinhibition at high irradiances was neglected in the photosynthetic models and photosynthesis was not integrated over depth. Surface photoinhibition has been known to limnologists and oceanographers for a very long time (Morel 1991, Falkowski *et al.* 1994, Miller 2006, Falkowski and Raven 2007) and as this study clearly shows, photoinhibition at high irradiances was a major limitation to the total P_N of shallow ponds (Figs. 1, 3, 4, 5). We found that the waiting-in-line model is capable of predicting photosynthesis at suboptimal, optimal and supraoptimal light (Ritchie 2008b), is easy to fit and gives better estimates of fitted parameters than the Eilers-Peeters equation (Eilers and Peeters 1988) or the Platt equation (Jassby and Platt 1976, McBride 1992, Ritchie 2008b). The pond P_N data shown in Table 2 and illustrated by Figs. 3, 4, and 5 for *Chlorella* all showed that photoinhibition in the first few mm depth of the pond resulted in little P_N in this layer. In the more strongly photoinhibited *Phaeodactylum*, net photosynthesis was negative at shallow depths

(Table 2). Grobbelaar *et al.* (1990) and Grobbelaar (2007) used a different light saturation and integration model to that used in the present study but drew similar conclusions.

The integrated-over-depth forms of the waiting-in-line equation can be used to adjust the pond depth so that there was optimum P_N with the sun at its zenith (Table 2; Figs. 3, 4, 5). A deeper pond would have to have a lower cell density otherwise many cells would not get enough light (Fig. 4) and the carbon product (the cells) would be more dilute and hence more expensive to harvest. Sukenik *et al.* (1991) found similar results for actual *Isochrysis* raceway pond cultures. This optimum depth is based upon knowledge of the characteristics of the photosynthesis *vs*. irradiance curves for the alga in question (waiting-in-line equation) and the light attenuating properties of the algal suspension (Eq. 4). It might seem that simple waiting-in-line P_G *vs*. E curves (Eq. 3: Appendix) would predict photoinhibition at high light intensities *i.e.* midday depression of photosynthesis. This is the case with single leaves but is not in the case of a stacked battery of photosynthetic surfaces (Ritchie 2010). For dense cultures approaching an optically black state, the integrated state is a simple saturation curve without photoinhibition at high light intensity (Ritchie 2010; *see* Appendix Eqs. 8 and 12). The light attenuation constants given in Table 2 show that the optimum pond depth is approximately the depth at which incident light was about 5 to 10 μmol m^{-2} s^{-1} PPFD or about 0.5% to 0.25% of full sunlight. This rule-of-thumb would be very useful in managing production ponds and could be automated using a colorimeter and a simple flow cell. For the various algae used in the present study, the gross productivity achievable on an incident surface area was of the order of 140 μg(C) m^{-2} s^{-1} [or ≈ 0.5 g(C) m^{-2} h^{-1}] for sunlight directly overhead when only ≈ 2% of incident irradiance was lost by reflection but for a realistic field situation the refractive/reflective properties of a pond surface has to be taken into account to estimate P_N during the course of a day (*see* Figs. 6*A,B*).

When the course of daily irradiance and the solar angle (for refraction calculation) are known it is possible, at least in principle, to calculate daily or yearly P_N for an algal pond (*e.g*. Grobbelaar *et al.* 1990). However, such calculations are more difficult than usually appreciated (Appendix, Eqs. 9–12); neglect of these complexities results in overestimation of the productivity of a pond (Fig. 6*B*). Hence the more experimentally based treatment in the present study has advantages over the purely theoretical approach of Engqvist and Sjöberg (1980) and McBride (1992). Table 3 gives estimates of total P_N for *Chlorella*, *Dunaliella* and *Phaeodactylum* ponds on the tropic of Cancer for the spring equinox, summer solstice, autumn equinox and winter solstice. Even the highest productivity found in the present study (for *Chlorella)* was only 4.773 ± 0.387 g(C) m^{-2} d^{-1} or since the optimum depth of the pond was 87 mm, only 54.9 ± 4.45 g(C) m^{-3} d^{-1} on a bulk volume basis: a value much lower than those often quoted in the literature (*e.g*. Kroon *et al.* 1989, Richmond and Zou 1999, MacIntyre *et al.* 2002, Grobbelaar 2007). The value for *Chlorella* was calculated for a cell suspension with about 2 g(Chl *a*) m^{-3} and an optimalised pond depth such that as much light as possible is used for photosynthesis. Such a pond is essentially an optically black object for blue and red light. If a denser culture was used it can be shown that the pond would need to be shallower to optimise total photosynthesis but the total photosynthesis of the water column would be about the same. Total productivity achievable in a pond situation is determined by the properties of the integrated form of the waiting-in-line function (Eqs. 7,8: Appendix). These properties govern gross photosynthesis *vs*. irradiance, photoinhibition in the uppermost layers, the light attenuating properties of the algal suspension and overall net photosynthesis is governed by the respiratory losses of fixed carbon by the cells of the entire water column. The highest productivity for the biotechnologically important *Dunaliella* was about one half that of *Chlorella* (Tables 2 and 3). In *Dunaliella* acclimated to high light there would be high levels of β-carotene (Borowitzka 1992, Sosik and Mitchell 1994). Although much of the β-carotene in such cells is not part of the light harvesting protein complexes, it would nevertheless effectively photoprotect or shade the photosynthetic apparatus under natural pond conditions.

Ponds need to be surprisingly shallow to optimise primary productivity, in the range of 0.1–0.5 m (Fig. 4) as acknowledged by some workers in the past (Westlake 1963, Larkum and Howe 1997, Borowitzka 1999, Grobbelaar *et al.* 1990, Richmond and Zou 1999). There are elementary practical engineering problems in maintaining large ponds of very shallow depths (Weissman *et al.* 1988, Kroon *et al.* 1989, Borowitzka 1999). Although it is possible to run large (≈ 5,000 m^2) ponds about 150 mm deep (Grobbelaar *et al.* 1990, Shimamatsu 1987, 2004), engineering problems probably preclude larger pondages of similar depth. Fig. 4 shows that for the density of *Chlorella* used in the present study a 0.3 m deep pond would give a suboptimal, though reasonable, P_N, but P_N would be very low for *Dunaliella* or *Phaeodactylum* ponds of such a depth. A much lower cell density (≈ 3.5 times lower) would need to be maintained for the optimum depth of a *Chlorella* pond to be about 300 mm. Another important parameter that needs to be taken into account in our approach is the degree of acclimation of the algae to high light. In a natural pond algae will be growing from day to day leading to self shading and different levels of photosynthetic production. We assumed that our algal cultures in the greenhouse would be in a semistable state due to rapid mixing and semicontinuous harvesting. The state of our algae can be assessed by their Chl density (Table 1). Variation around these values would make little difference to the values of pond production that we predicted. However, it must be

pointed out that motile algae such as *Dunaliella* and algae able to adjust their buoyancy can avoid unfavourable light regimes and so using such species higher production could be achieved in ponds deeper than those based upon our calculations. Essentially a population of mobile algae would self-assemble into a layer of cells that would use the light regime in an optimal manner.

It can be difficult to grasp the problems of scale involved in primary production using an algal suspension. Consider that one tonne of algal suspension with a production rate of ≈ 55 g(C) m^{-3} d^{-1} in a pond 87 mm deep would occupy an area of 11.5 m^2. Not only are very large areas required for useful production of fixed carbon but there is also a volume problem. A pond able to fix 1 metric tonne (t) of carbon in one day would need to have an area of 21 ha and would contain 18,000 t of pondwater. In terms of production per bulk volume this is only 0.055 ppm d^{-1}. This underscores the fact that algal cultures are a very dilute source of organic carbon compared to a terrestrial crop (Borowitzka 1992, 1999, Giordano and Beardall 2009). The amount of carbon fixed per unit area is important from the point of view of production but in terms of economic processing and harvesting the amount of product per unit volume is more critical.

A rough estimate of annual P_N (as tonnes of carbon per hectare per year) could be made from the daily productivities calculated for each season (Table 3). For a *Chlorella* pond located at the tropic of Cancer, P_N would be approximately 14.1 ± 0.66 t(C) ha^{-1} y^{-1}; for *Dunaliella,* 5.48 ± 0.39 t(C) ha^{-1} y^{-1} and for *Phaeodactylum,* 6.58 ± 0.42 t(C) ha^{-1} y^{-1}. All such calculations are estimates of *potential production* because they do not take into account weather such as cloud cover or temperature. Perhaps more crucially, Fig. 6*B* shows that the refraction/reflectance properties of the pond surface had virtually unavoidable negative effects on P_N in the mornings and afternoons when the solar angle was low (Eqs. 9–12).

It is inappropriate to estimate P_N in a pond as if the sun was continuously directly overhead and to assume that only the intensity of the irradiance varies over the course of the day. If this assumption is made P_N is overestimated by 4% for the *Chlorella* summer solstice data shown in Fig. 6*B*. For any particular algal culture, the total integrated P_N of a pond depends mainly upon the surface area of the pond and the optimal depth of the pond for the incident light available (Richmond and Zou 1999, Grobbelaar *et al.* 1990, Grobbelaar 2007). Daily P_N is also negatively affected by the solar angle. This effect would be more severe for ponds located at higher latitudes than at the Tropic of Cancer because of the lower solar angles even at the summer solstice. This does not appear to have been emphasised before. It is worth pointing out here that floating plants such as water lilies (Ritchie 2012) and even buoyant algae avoid most of the negative consequences of refraction in the water column.

Our model makes a number of simplifying assumptions and under real conditions the growth rate is likely to be lower for many different reasons (Smayda 2006). Some of the most important assumptions and limitations are:

(*1*) No account has been taken of shading by clouds. As a rule cloud-cover reduces irradiance by about 1/3 (Ritchie 2010). Cloud cover could reduce annual production considerably depending on the curvature of total photosynthesis *vs*. E curves (Fig. 5).

(*2*) We have assumed that the ponds are homogeneous. We have not concerned ourselves in detail how this would be achieved or its energy costs. Shimamatsu (2004) points out that the energy costs of circulation of algal production ponds is a matter of intellectual property and so detailed information is often not readily available.

(*3*) We have assumed that it is possible to extrapolate from greenhouse cultures to pond conditions.

(*4*) We have assumed that nutrient supply is optimal and nonlimiting: only light is the limiting factor. We have assumed an appropriate temperature of the growth medium, which in a shallow pond during the middle of a summer day would be very difficult to control (Fawley 1984, Dauta *et al.* 1990, Sosik and Mitchell 1994, Borowitzka 1999).

(*5*) We have assumed an adequate and cheap water supply to replenish evaporation is available (a consideration that is often neglected in proposals to set up production ponds in arid climates).

(*6*) We have assumed lack of pathogens or growth inhibitors or invasive zooplankton herbivores such as *Daphnia*. Of the three algae used in the present study only *Dunaliella* lives in a habitat that effectively eliminates herbivory and competitors (Borowitzka 1999).

(*7*) On the very large scales envisaged for biofuels production, CO_2 enhancement would not be practicable except perhaps in the case of algal ponds associated with thermal power plants.

(*8*) The negative effects of UV radiation on photosynthesis have probably been underestimated in the present study (Davidson 2006). The algae were grown in a glasshouse (ordinary glass is UV opaque). Neither the PAM machine nor the oxygen electrode setup used in the present study measured photosynthesis in the presence of significant UV light.

(*9*) In the present study we have attempted to estimate Net Photosynthesis (P_N) of pondages. Primary production of useable carbon product in real situations would be considerably less than P_N because of loss of fixed carbon as photorespiratory dissolved organic carbon (DOC) and its metabolism by microbes and so the O_2/C ratio is greater than unity (Falkowski *et al.* 1994, Larkum *et al.* 2003, Falkowski and Raven 2007).

(*10*) In mid-latitudes primary production would be very low or zero in winter. For example, the Earthrise® *Spirulina* farm at Calipatria, California (33.199312ºN, 115.559698ºW) is stated to have a 7-month production

season (Shimamatsu 2004). Predictably enough, Grobbelaar *et al.* (1990) found minimal production in *Scenedesmus* ponds in Germany during winter.

(*11*) Pondage algal production is basically growing and harvesting an algal bloom and so it has more in common with cropping than growing algae in a chemostat. True continuous harvesting might not be possible and in practical situations growth and harvesting of algae in ponds are more likely to resemble semicontinuous culture regimens (Borowitzka 1999). Algal ponds will have a significant "down-time" even in climates where year-round growth is theoretically possible.

Highly productive systems such as coral reefs, rainforests, seagrass and kelp beds and highly productive field crops during their optimum growth phase, *e.g.*, sugarcane (*Saccharum* spp.), corn (*Zea mays*), and cotton (*Gossypium barbadense*) are capable of seasonally sustained P_N rates of up to 10–12 g(C) m^{-2} d^{-1} (Colinvaux 1978, Larkum *et al.* 2003, Robertson *et al.* 1996, Miller 2006, Falkowski and Raven 2007). These P_N rates are more than twice the best P_N found in the present study (Table 3, *Chlorella*, P_N = 4.773 ± 0.387 g(C) m^{-2} d^{-1}). Land plants are able to maintain a high P_N because they are homoiohydric (*i.e.*, obtain their water supply from roots) (Larkum 2010) and take advantage of the much higher rate of diffusion of gases in air, in contrast to water, to source their CO_2 from the atmosphere. The only aquatic communities that can match this are either very shallow (ponds and soda lakes of Ethiopia – *see* below), coastal kelps and seagrasses stirred by wave action and coral reefs that maintain thin algal communities, and which are constantly turned over by grazing and so there is little build-up of organic carbon. The values for all these systems are not very far short of the theoretically maximum for P_G for photoautotrophs lacking phycobilin pigments (≈ 16 to 18 g(C) m^{-2} d^{-1}; Ritchie 2010): In line with this, Talling *et al.* (1973) found productivities as high as 16–21 g(C) m^{-2} d^{-1} in soda lakes in Ethiopia dominated by *Spirulina,* a cyanobacterium, that can utilise a larger proportion of the PPFD spectrum than photoautotrophs without phycobilin pigments (Ritchie 2010). Many of the other claims for carbon production well above 20 g(C) m^{-2} d^{-1} in algal ponds and photobioreactors would seem to be thermodynamically impossible (Ritchie 2010). On the other hand, Richmond and Zou (1999) and Grobbelaar (2007) offer estimates of no more than 10 g(C) m^{-2} d^{-1} for *Spirulina,* which are more in line with the present calculations. Many of the higher values only apply to high summer and for short (often unspecified) periods of time. If calculated on an annual basis carbon fixation would probably fall to considerably less than 10 g(C) m^{-2} d^{-1} [< 36.5 t(C) ha^{-1} y^{-1} or < 36.5 t(C) ha^{-1} y^{-1}]. It might be argued that the reason that our estimates of production are low is because of the extrapolation from greenhouse cultures to pond conditions. However, we have developed an internally consistent model for estimating production in ponds and have shown that a culture thick enough to absorb all light has an inherently limited productivity (*see* Appendix and Ritchie 2010). Also note that we have used an optimal temperature throughout: taking daily temperature changes into account (where suboptimal temperatures would be experienced in any real system) leads only to lower production (Fawley 1984, Dauta *et al.* 1990, Sosik and Mitchell 1994).

There seems to have been a long-standing and persistent idea that algae are inherently highly productive as illustrated by many recent publications (Sheehan *et al.* 1998, Moheimani and Borowitzka 2006a,b; Huntley and Redalje 2007, Chisti 2007, 2008a,b; Weyer *et al.* 2010). The idea that algae are highly productive compared with land plants is a fallacy that can be dated back to Warburg (1919) and is still widely believed (Colinvaux 1978, Belasco 1997, Weyer *et al.* 2010), particularly in the form of the fable that a few litres of algal culture will provide enough O_2 for an astronaut (Ai *et al.* 2008). Comparison of photosynthetic rates of algae and a benchmark vascular plant species (pea) in Table 1 clearly showed that this was not so. Waltz (2009) points out that opinions to the contrary by researchers such as Walker (2009, 2010), Larkum (2010) and Ritchie (2010) who work on the biophysics of photosynthesis, are not properly considered. The basic problems are that (*1*) light is a dilute energy source, (*2*) only part of the 400–700 nm spectrum is actually used by photosynthesis (about 35–40%, Ritchie 2010), (*3*) photosynthesis has limited thermodynamic efficiency, (*4*) this thermodynamic efficiency decreases with increasing irradiance and the theoretical asymptotic photosynthetic efficiency (α) calculated at zero irradiance is often misused, (*5*) stirring can be a severe limitation in many aquatic systems, and (*6*) algal suspensions are very dilute on a w/w basis. It is true that theoretically algae can be grown in photobioreactors at higher rates than crop plants and are not hampered by annual growth cycles. However, as shown here, the major problem in culturing algae is to match the light intensity to the optimum for concentrated algal suspensions and to avoid (*1*) photoinhibition or (*2*) light levels below the compensation point. Many other practical problems such as stirring, nutrient supply, water supply, harvesting, and pathogens, make algal cultures far less attractive in practice than in theory. In most photobioreactors the same problems exist as with ponds. For photobioreactors the energy input for stirring would likely be far greater than for ponds and this would compromise the desired small solar footprint of the designs (Larkum 2010). For bulk production of carbon the open raceway pond remains the only practical method of producing a low cost bulk product (Weissman *et al.* 1988, Grobbelaar 2007) but our study has highlighted its limitations.

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Appendix

The fluorescence yield was calculated using the *WinControl* software as the effective quantum yield (Φ_{PSII}) as defined by Genty *et al.* (1989). The range of light intensities was adjusted so that, if possible, the optimum light was near the mode of the range of light intensities used. ETR is an estimate of gross photosynthesis and is defined as:

$$rETR = \Phi_{PSII} \times E \times 0.5 \times 0.84 \quad (1)$$

where Φ_{PSII} is the effective quantum yield, E is the irradiance [μmol m^{-2} s^{-1} PPFD], 0.5 is the PSI/PSII allocation factor allowing for about 50% of quanta being absorbed by PSII (Melis 1989) and 0.84 is the leaf absorptance constant which is the mean absorptance factor for land plants (Björkman and Demmig 1987, Genty *et al.* 1989). It should be noted that this leaf absorptance constant is now known to be more variable than previously believed (Stemke and Santiago 2011). The default value for the ETR absorption factor (0.84) is a reasonable approximation to use for a glass fibre disk impregnated with a thick layer of algae (Table 1) but because we did not actually measure the absorptance of the disks the ETR quoted is designated the relative electron transport rate (rETR). [Subsequent unpublished experience with a prototype absorptance meter has shown that glass fibre disks impregnated with *Chlorella* cells to a density of about 50 mg(Chl *a*) m^{-2} have absorptances of about 0.8 to 0.9 in blue light (≈ 465 nm). Hence there was little error generated in the present study by using the 0.84 standard absorptance value and so the actual ETR ≈ rETR].

The optimum irradiance (E_{opt}) and the shape of light curves are not affected by the values of the allocation factor or the leaf/ culture absorptance constant. They affect the calibration of ETR with oxygen evolution or carbon fixation. Four electrons are moved through PSII for each O_2 produced in photosynthesis and so an ETR of 4 μmol(e^-) m^{-2} s^{-1} is equivalent to an approximate P_G of 1 μmol(O_2) m^{-2} s^{-1} in terms of oxygen (O_2) evolution (Ritchie 2008b, 2012; Ritchie and Bunthawin 2010a, b).

rETR values were plotted as light-response curves (P_G *vs*. E). It follows from the finding that plots of Φ_{PSII} *vs*. E obey a simple exponential decay function (Gloag *et al.* 2007, Ritchie 2008b, Ritchie and Bunthawin 2010a,b) that plots of rETR *vs*. E should obey an exponential function known as the waiting-in-line model (probability density function or exponential waiting time distribution) (Steele 1962, Gloag *et al.* 2007, Ritchie 2008b, Ritchie and Bunthawin 2010a,b; Ritchie 2012). The waiting-in-line equation is,

$$y = x\, e^{-x} \quad (2)$$

Eq. 2 has a maxima ($dy/dx = 0$) at $x = 1$, the slope of the line at $x = 0$ is 1 and there is a point of inflection ($d^2y/dx^2 = 0$) at $x = 2$. A form suitable for modelling photosynthesis (Gloag *et al.* 2007, Ritchie 2008b) that is easy to fit using non-linear least squares methods (Ritchie and Bunthawin, 2010a,b; Ritchie 2010, 2012) is,

$$P_G = \frac{P_{Gmax}\, E}{E_{opt}}\, e^{1-E/E_{opt}} \quad (3)$$

where, P_G is gross photosynthesis measured as rETR, O_2 evolution or CO_2 uptake, E is the irradiance (μmol m^{-2} s^{-1} 400–700 nm PPFD), E_{opt} is the optimum irradiance, and P_{Gmax} is the maximum gross photosynthesis, E_{opt} is equivalent to $1/k_w$ and A was a Y-axis scaling constant ($A = e \times P_{Gmax}$) in previous versions of Eq. 3 (Gloag *et al.* 2007, Ritchie 2008b). The maximum photosynthetic efficiency (α) is the initial slope of the curve at $E = 0$ ($\alpha_0 = e \times P_{Gmax}/E_{opt}$). At very low light intensities photosynthesis is directly proportional to irradiance. The half-maximum photosynthesis ($P_{G,\,half\text{-}max}$) occurs at 0.23120 times E_{opt} and photosynthesis is inhibited by 50% at 2.6734 times E_{opt} (Ritchie 2008b). Eq. 3 has only two unknowns and is easy to fit. This is a better model of P_G *vs*. E than models requiring the determination of three unknowns such as a simple rectangular hyperbola with a term added to take photoinhibition into account (Grobbelaar *et al.* 1990), the exponential difference equation (Platt equation: Jassby and Platt 1976, McBride 1992) or the Eilers and Peeters (E-P) equation (Eilers and Peeters 1988).

Optical transmission of algal cultures was measured using a Taylor Sphere attachment (*ISR-240A*) fitted to a *Shimadzu UV-2550* UV-visible spectrophotometer using quartz cuvettes. Lamberts law adequately described attenuation of the blue absorption maxima *vs*. depth (Grobbelaar *et al.* 1990, Ritchie 2010),

$$E_x = E_0\, e^{-k_L x} \quad (4)$$

where E_x is the irradiance at depth x [μmol m^{-2} s^{-1} PPFD], E_0 is the irradiance at the surface of a pond with the sun directly overhead (full sunlight is ≈ 2,200 μmol m^{-2} s^{-1} PPFD, *see* Ritchie (2010), k_L is the attenuation of irradiance with depth [m^{-1}], and x is the vertical depth [m].

P_G and P_N at depth x of an algal pond in sunlight can now be modelled. Taking the waiting-in-line equation (Eq. 3) and substituting E with the equation for E *vs*. depth (Eq. 4),

$$P_G = e\ P_{Gmax}\ k_w\ E_0/E_{opt}\ e^{-k_L x - E_0/E_{opt}\ e^{-k_L x}} \tag{5}$$

For P_N at depth x a respiration term (R) needs to be included,

$$P_N = e\ P_{Gmax}\ E_0/E_{opt}\ e^{-k_L x - E_0/E_{opt}\ e^{-k_L . x}} + R \tag{6}$$

where P_{Gmax} is the maximum gross photosynthesis [a constant expressed as mol(O_2) mg^{-1}(Chl*a*) h^{-1} or mol(O_2) m^{-3} s^{-1}], E_0 is the irradiance normal (90º) to the surface, E_{opt} is the optimum irradiance (as for Eq. 3), k_L is the light attenuation coefficient of the algal suspension (as for Eq. 4), R is the respiration rate [mol(O_2) mg(Chl *a*)$^{-1}$ h^{-1} or mol(O_2) m^{-3} s^{-1}], and x is the vertical depth [m].

For practical situations usually only P_N would be of interest in an algal pond. P_N (Eq. 6) includes a term allowing for respiration (R). The integral of total P_N from the surface (x = 0) to depth x is,

$$\sum_0^x P_N = \int_0^x e\ P_{Gmax}\ E_0/E_{opt}\ e^{-k_L x - E_0/E_{opt}\ e^{-k_L x}} + R\,dx = \left[\frac{e\ P_{Gmax}\ e - E_0/E_{opt}\ e^{-k_L x}}{k_L} + R\ x \right]_0^x + C \tag{7}$$

where C is the constant of integration, P_{Gmax}, k_L, E_{opt}, E_0, R, and x have the same meanings as above.
After evaluating C, the final form of the equation describing total P_N for a pond with a vertical depth of x is,

$$\sum P_N = \left[\frac{e\ P_{Gmax}\ e^{-E_0/E_{opt}\ e^{-k_L x}}}{k_L} + R\,x \right] - \left[\frac{e\ P_{Gmax}\ e^{-E_0/E_{opt}}}{k_L} \right]$$

or re-arranging,

$$\sum P_N = \frac{P_{Gmax}}{k_L}(e^{1 - E_0/E_{opt}\ e^{-k_L x}} - e^{1 - E_0/E_{opt}}) + R\ x \tag{8}$$

where the constant P_{Gmax} and R are best expressed in terms of mol(O_2) m^{-3} s^{-1} and k_L and x need to be expressed as m^{-1} and m respectively, giving estimates of the integrated P_N ($\sum P_N$) in terms of the surface area of the pond [mol(O_2) m^{-2} s^{-1}] and for a specified depth of the pondage (x). P_G for a pond of depth x can be calculated from Eq. 8 by setting R at zero. Respiration of a water column of the algal suspension in darkness is simply the product of R [mol(O_2) m^{-3} s^{-1}] and the depth of the water column [x in metres]. Eq. 8 is essentially equivalent to the integrated form of the waiting-in-line equation derived by Engqvist and Sjöberg (1980).

Three pieces of information are required to calculate irradiation of a pond during the course of a day. The irradiance and angle of attack of the sunlight over the course of the day was calculated for a horizontal surface using the *SMARTS* software (*SMARTS* 2009) as described by Ritchie (2010). *SMARTS* corrects solar irradiance for refraction and the absorption effects of the thickness of the atmosphere through which sunlight passes. Refraction, reflection and absorption are most severe at low solar angles.

At the water surface refraction and reflection have two effects on incident irradiance: a proportion of incident irradiance is reflected off the pond surface, the remaining light is bent towards the normal (perpendicular). Firstly, calculating the incidental light angle normal to the pond surface from the solar elevation angle,

$$\theta_i = 90 - \beta_i$$

where β_i is the solar angle to the horizon and θ_i is the incident angle normal to the pond surface.
Snell's law can then be used to calculate the angle of transmission (Lorrain *et al.* 1988).

$$\frac{\sin\theta_i}{\sin\theta_t} = \frac{n_t}{n_i} \text{ or solving for } \theta_t,$$

$$\theta_t = \arcsin\left(\frac{\sin\theta_i\ n_i}{n_t}\right) \text{ or } \theta_t = \arcsin\left(\frac{\cos\beta_i\ n_i}{n_t}\right) \tag{9}$$

where the angle θ_i refers to the normal incident ray ($90 - \beta_i$), the angle θ_t refers to the angle normal to the surface of the transmission medium, n_i is the refractive index of the incident medium (i) (in this case air) and n_t is that of the transmission medium (t) (the pondwater), (i) is incident light and (t) is transmitted light.

By trigonometric construction the relative light path of a ray bent by refraction in the pondwater is,

$$x_{rel} = \frac{1}{\cos\theta_t} \text{ or } \frac{1}{\cos\left(\arcsin\left(\frac{\cos\beta_i\, n_i}{n_t}\right)\right)} \tag{10}$$

where x_{rel} is the relative change in the relative light path compared to the unit depth of the pond.

The proportion of incident light penetrating the pond can be calculated from Fresnel's equation for unpolarised light (Lorrain *et al.* 1988),

$$E_{trans} = 1 - 0.5 \times \left(\frac{n_i \cos\theta_i - n_t \cos\theta_t}{n_i \cos\theta_i + n_t \cos\theta_t}\right)^2 - 0.5 \times \left(\frac{n_i \cos\theta_t - n_t \cos\theta_t}{n_i \cos\theta_t + n_t \cos\theta_i}\right)^2 \tag{11}$$

where E_{trans} is the proportion of incident irradiance that is transmitted into the pond.

Tables of light transmission, refraction and relative light path *vs*. solar angle for freshwater, seawater and *Dunaliella* brine at 25ºC are provided as supplementary material.

Eq. 8 can now be modified to take into account the refractive and reflective effects at the pond surface reducing the irradiance penetrating the water surface ($E_{rel}.E_o$) and the length of the light path corrected for refraction ($x_{rel}.x$),

$$\sum P_N = \frac{P_{Gmax}}{k_L}\left(e^{1 - E_{rel}\, E_0/E_{opt}\, e^{-k_L\, x_{rel}\, x}} - e^{1 - E_{rel}\, E_0/E_{opt}}\right) + R\, x \tag{12}$$