

Protein turnover in relation to maintenance metabolism at low photon flux in two marine microalgae

A. QUIGG* & J. BEARDALL

School of Biological Sciences, Monash University, Clayton, Victoria, 3800, Australia

ABSTRACT

Acclimation to very low photon fluxes involves adjusting a suite of physiological characteristics that collectively elicit a physiological response. Facilitating such changes is protein turnover. *Dunaliella tertiolecta* (Butcher) and *Phaeodactylum tricornutum* (Bohlin) were grown in turbidostats at a range of photon fluxes between 2 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The kinetics of pulse-chase labelling of the protein with ^3H showed that (1) two protein pools were present, one of which turned-over rapidly (hours), and a second which turned over more slowly (days); and (2) protein turnover rates were slower in *P. tricornutum* than in *D. tertiolecta*. *Phaeodactylum tricornutum* had a lower maintenance coefficient for protein turnover than *D. tertiolecta*, and correspondingly a smaller proportion of its respiratory demands (30%) were associated with protein turnover than in *D. tertiolecta* (36%). There appears to be a correlation between lower metabolic activity, requiring lower protein concentrations, and an associated decreased cost of maintenance processes in *P. tricornutum* compared to *D. tertiolecta*. Differences between protein turnover rates and maintenance metabolic costs may be one of the photo-acclimation strategies that determine which photon niches microalgae can successfully exploit.

Key-words: *Dunaliella tertiolecta*; *Phaeodactylum tricornutum*; maintenance metabolic rate; photo-acclimation; protein turnover; respiration.

INTRODUCTION

Major limitations on photolithotrophic growth at extremely low photon fluxes are thought to be protein turnover, charge recombination in photosystem II, and proton leakage and slippage (Raven, Külber & Beardall 2000). These processes act synergistically, limiting rates of photosynthesis, and affecting the minimum specific growth rate that eukaryotic microalgae can sustain. Protein turnover will be

defined as a cycle of degradation and re-synthesis, and is the focus of the present investigation. This essential process allows cells to re-utilize amino acids, to exchange protein content during growth, and to acclimate to environmental stimuli (Huffaker & Peterson 1974; Davies 1982; Vierstra 1993). Protein turnover is believed one of the most significant components of maintenance processes (and hence dark respiration) in terms of total cell energy requirements (Penning de Vries 1975; Raven & Beardall 1981; Geider & Osborne 1989; De Visser, Spitters & Bouma 1992; Zagdańska 1995; Geider, MacIntyre & Kana 1996; Lambers, Chapin & Pons 1998; Raven *et al.* 2000). This has important implications for plant carbon balance, as any factor that increases protein concentration will significantly increase maintenance costs. The amount of quantitative data on protein turnover as a function of maintenance costs for higher plants is increasing (Van der Werf *et al.* 1992; De Visser *et al.* 1992; Bouma *et al.* 1994; Zagdańska 1995; Scheurwater *et al.* 2000). However, for algae, the reports are scarce (Richards & Thurston 1980a,b; Thurston & Richards 1980).

Growth of marine microalgae is likely to be frequently light-limited, particularly during intense mixing of the euphotic zone and in the deep-water chlorophyll maximum layer. The diatom *Phaeodactylum tricornutum* has been shown to grow at $\leq 1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Geider, Osborne & Raven 1985, 1986). *Dunaliella tertiolecta*, a chlorophyte, exhibits 20-fold higher compensation photon fluxes for growth (I_{cg}) (Falkowski & Owens 1980; Quigg 2000). The parameter I_{cg} defines the point at which photosynthesis and respiration rates are in balance, so that the net growth rate is zero. One of the major differences between high- and low- I_{cg} microalgae is thought to be their maintenance costs (Geider *et al.* 1985). Maintenance respiration rates can be used to estimate (assuming complete coupling in respiratory energy conversions) the energy available for maintenance processes. The likelihood that some of the maintenance energy requirements in the light are supplied by the direct use of photoproduced adenosine triphosphate (ATP) rather than via respiration cannot be ignored, but is difficult to quantify and would have little effect on the overall energy budget (Raven & Beardall 1981). In addition, Bouma *et al.* (1994) suggested that many of Penning de Vries (1975) calculations need to be reviewed in the light of new information on the process(es) associated with protein (re)synthesis.

Correspondence: A. Quigg. Fax: +1 732 932 4083; e-mail: aquigg@imcs.rutgers.edu

*Present address: Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers, The State University of New Jersey, 71 Dudley Road, New Brunswick, New Jersey, 08901, USA.

Given this, we have quantified the rates of protein turnover in *P. tricornutum* and *D. tertiolecta* using a ^3H labelling and dilution (pulse-chase) method modified for use with microalgae. The energy demand of the measured rate of protein turnover was then compared with the energy available for maintenance processes under the same conditions. Using this information, the fraction of maintenance energy associated with protein turnover was estimated. Overall, maintenance costs for *D. tertiolecta* were significantly higher than those of *P. tricornutum*. These relationships were considered in relation to the different photo-acclimation strategies used by *D. tertiolecta* and *P. tricornutum*, and their effect on the minimum photon flux for photolithotrophic growth.

MATERIALS AND METHODS

Growth conditions

Dunaliella tertiolecta (CS-175) and *P. tricornutum* (CS-29) were obtained from the CSIRO Culture Collection of Microalgae (CSIRO Division of Oceanography, Tasmania, Australia) and grown in 500 mL turbidostats in the artificial seawater medium, PHK (a modified 'D' medium; Provasoli, McLachlan & Droop 1957). The cultures were grown under 24 h continuous illumination at 18 °C for a minimum of 5–10 generations (depending on the growth rate) at seven photon fluxes between 2 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

For the protein turnover experiments, *D. tertiolecta* and *P. tricornutum* were grown at four photon fluxes corresponding to (1) a photon flux close to their I_{cg} that will be designated μ_{min} , where the minimum growth rate was measured; (2) two growth rates corresponding to 25 and 50% of the maximum growth rate; and (3) the maximum growth rate (μ_{max} or 100%) (Table 1).

Protein turnover experiments were not carried out directly in turbidostats (due to constraints on equipment and a requirement to minimize the amount of $^3\text{H}_2\text{O}$ used), but in smaller (20 mL) chambers. These included an air inlet to aerate and mix cultures and an air outlet that was passed through to a desiccated silica gel trap. During chase experiments, cells were kept in the mid-exponential growth phase by periodic dilutions with fresh PHK medium. This ensured that cultures were neither nutrient-limited nor self-shading. Cell biomass was monitored at 24-hourly intervals by measuring *in vivo* chlorophyll (Chl) fluorescence with a Hitachi 2000 spectrofluorometer (Hitachi Australia Ltd, North Ryde, NSW, Australia), using excitation and emission wavelengths of 430 and 680 nm, respectively.

Gas exchange measurements

Samples drawn from turbidostats were used to prepare photosynthesis versus irradiance curves for *D. tertiolecta* and *P. tricornutum*. A Clark-type Hansatech DW1 oxygen electrode (Hansatech Instruments Ltd, Kings Lynn, Norfolk, UK) was used to measure rates of dark respiration

(R_d), followed by rates of O_2 evolution at 12 increasing photon fluxes between 2 and 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, at 18 °C. A slide projector fitted with a quartz halogen globe and appropriate neutral density filters was used for illumination. The photon flux incident on the internal surface of the electrode chamber was measured using a Li-Cor Integrating Quantum Radiometer/Photometer (Li 188B; LiCor Inc., Lincoln NE, USA). Cells were exposed to each photon flux for 3–5 min, by which time rates of O_2 exchange had stabilized. Photosynthesis versus irradiance data were modelled using the equation of Platt, Gallegos & Harrison (1980), and normalized to cell number, cellular carbon or Chl *a*. Mean O_2 exchange rates (\pm SE) were then estimated from a minimum of six replicate curves performed on cells grown at each photon flux (Table 1). A Superior Improved Neubauer haemocytometer (ProSciTech, Thuringowa, QLD, Australia) was used for cell counts. Cellular carbon and nitrogen were determined on the same cultures ($n \geq 3$), dried onto Whatman GF/C filters (Whatman International Ltd, Maidstone, Kent, UK), and analysed with a Carlo ERBA CHN analyser (ThermoFinnigan, Hemel Hempstead, Herts, UK). Pigments were extracted with 90% acetone; Chl *a* was then determined using the equations of Jeffrey & Humphrey (1975).

The ratio of dark respiration rate to maximum photosynthetic rate ($R_d : P_m$) has been previously reported to be a sensitive indicator of the physiological state of algal cells (Verity 1982; Geider & Osborne 1989; Falkowski & Raven 1997). During protein turnover experiments, R_d and P_m were measured in cells growing in both turbidostats and chambers. R_d was measured first, followed by P_m once cells had been illuminated at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 7–10 min. Photosynthesis versus irradiance curves had established this photon flux as saturating for O_2 evolution, but not photo-inhibitory.

Pulse-chase experiments

Protein turnover was measured by modifying the $^3\text{H}_2\text{O}$ labelling method of Humphrey & Davies (1975, 1976) for use with microalgae. A reverse isotope dilution in which $^3\text{H}_2\text{O}$ was incorporated into the protein pools (pulse experiment – 24 h) and an isotope dilution, in which labelled proteins were chased with endogenous/exogenous H_2O (chase experiment) was performed. *Dunaliella tertiolecta* and *P. tricornutum* suspensions were divided into five chambers: three were inoculated with 18.5 kBq mL^{-1} $^3\text{H}_2\text{O}$ and two were unlabelled (controls). Aliquots were removed hourly to monitor the time course of ^3H incorporation into proteins and growth (Fig. 1). Both the protein content (see below) and proportion of labelled protein [via liquid scintillation counting with aqueous counting scintillant II (ACS II); Amersham Biosciences Pty, Ltd, Castle Hill, NSW, Australia] were measured. After the pulse, unincorporated label was removed from cells with three washes (10 mL) of fresh PHK medium (2500 \times g, 10 min). Cells were re-suspended in fresh medium to start the chase

Table 1. Cellular composition and photosynthetic characteristics of *D. tertiolecta* and *P. tricornerutum* as a function of the photon flux for growth

Organism	Photon flux for growth ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	Growth rate (μ) as a function of μ_{max} ^a	P_m^C ($10^{-5} \text{ mol O}_2 \text{ mol C}^{-1} \text{ s}^{-1}$)	C : N (mol C mol ⁻¹ N)	Chl a : C (mg Chl a : mg ⁻¹ C)	Protein pool size (pg protein cell ⁻¹)
<i>D. tertiolecta</i>						
	30	μ_{min}	1.33 (0.08)	5.16 (0.86)	0.069	15.8 (2.7)
	45	25%	2.20 (0.09)	6.49 (0.16)	0.066	8.75 (8.0)
	70	50%	2.94 (0.16)	8.85 (0.05)	0.084	8.24 (1.9)
	100		6.34 (0.45)	4.90 (0.14)	0.103	
	130	μ_{max} (100%)	6.06 (0.55)	3.01 (0.17)	0.072	8.12 (2.2)
	220		2.33 (0.36)	4.32 (0.17)	0.041	
	285		1.66 (0.13)	5.07 (0.33)	0.033	
<i>P. tricornerutum</i>						
	2.5	μ_{min}	2.14 (0.09)	nd	0.075	3.85 (0.4)
	6	25%	2.18 (0.15)	5.11 (0.11)	0.046	2.73 (0.6)
	15	50%	2.29 (0.13)	5.77 (0.18)	0.045	3.49 (2.1)
	45		2.33 (0.24)	5.14 (0.13)	0.034	
	85		1.72 (0.07)	5.38 (0.12)	0.056	
	100	μ_{max} (100%)	1.83 (0.08)	6.21 (0.08)	0.021	2.34 (0.5)
	140		3.04 (0.18)	nd	nd	

^aRefer to text for further details.

Values are averages (\pm SE). n.d., not measured.

experiment. Aliquots were again removed at regular intervals.

Although the data fitted a two-component first-order decay model well in many cases ($r^2 > 0.95$), it was more realistic to regard the classes of protein in the samples as a heterogenous assembly where the observed decay rates were average values of a range of individual rates. The rate constant for protein degradation (k_d ; d⁻¹) was the negative of the slope of a plot of the natural log of ³H-protein activity against time. The rate constants for *D. tertiolecta* and *P. tricornerutum* were corrected for growth rate dilution by subtracting the growth rate ($k_d = \text{rate constant} - \mu$). Three independent pulse-chase experiments (as a minimum) were performed at each growth rate for both species.

Determination of total protein concentration

Cell samples were thawed on ice, vortexed, and then centrifuged ($15\,000 \times g$, 5 min). The supernatant was discarded, the pellet washed, and the cells re-suspended in fresh medium. For *D. tertiolecta*, this step was repeated to remove extracellular material. *Phaeodactylum tricornerutum* cells were disrupted with a Branson sonicator (Branson Ultrasonic Corporation, Danbury, CT, USA) (2×15 s bursts), on ice, and centrifuged ($15\,000 \times g$, 5 min). Pellets were then re-suspended in ice-cold acetone (90%) and kept on ice for 30 min to extract pigments. Samples were then centrifuged ($15\,000 \times g$, 5 min), the supernatant discarded, and the pellets air-dried. These were then re-suspended in 500 μL 0.1 M

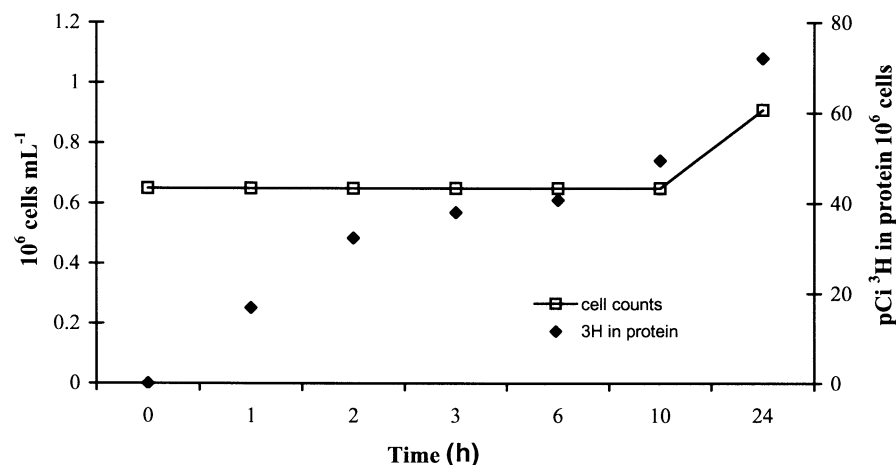


Figure 1. Time course of ³H incorporation into proteins (right-axis) was monitored over a 24 h pulse-experiment. Cell counts (left-axis) were used to follow growth. This is a representative figure, taken from an experiment on *D. tertiolecta* grown at 45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (25% μ_{max}).

NaOH/1% sodium dodecyl sulphate, incubated at 100 °C for 5 min, and then centrifuged (15 000 × g, 5 min). Protein in the supernatant was assayed at 750 nm according to Peterson (1977) using a bovine serum albumin standard curve as a reference.

RESULTS

Response of the various physiological parameters to the photon flux for growth

Growth rates of *D. tertiolecta* and *P. tricornutum* (Fig. 2a & b, respectively) exhibited a curvilinear response with photon flux for growth. Maximum growth rates were 1.4 and 1.15 d⁻¹ for *D. tertiolecta* and *P. tricornutum*, respectively. I_{cg} was calculated using the first three linear points on the growth curves, giving values of 18 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *D. tertiolecta* and 2.4 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *P. tricornutum*.

Carbon-specific dark respiration rates (R_d^C) and maximum photosynthetic rates (P_m^C) increased linearly with growth rate in *D. tertiolecta*, up to approximately

200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 3a; Table 1). R_d^C and P_m^C did not change as a function of the photon flux used to grow *P. tricornutum* (Fig. 3b; Table 1). The Chl *a* : C ratio (Table 1) and C and N quotas (Fig. 4a & b) decreased with the photon flux for growth in *D. tertiolecta* and *P. tricornutum*. Small increases in the C and N quotas, however, were seen at photon fluxes >200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *D. tertiolecta* (Fig. 4a) and >100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *P. tricornutum* (Fig. 4b). Despite variations in C and N concentrations, the average C : N ratio was $5.29 \pm 1.76 \text{ mol C mol}^{-1} \text{ N}$ for *D. tertiolecta* and $5.53 \pm 0.42 \text{ mol C mol}^{-1} \text{ N}$ for *P. tricornutum* (Table 1). Total protein concentration in *P. tricornutum* ($3.10 \pm 0.8 \text{ pg protein cell}^{-1}$) did not change as a function of the photon flux for growth (Table 1). Only in *D. tertiolecta* growing close to their I_{cg} (30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was there a noticeable increase in the size of the protein pool (Table 1). At all other photon fluxes for growth, the protein pool of *D. tertiolecta* was some 53% smaller (8.37 pg protein cell⁻¹). Differences in the physiological parameters (R_d^C , P_m^C , protein pool) of *D. tertiolecta* and *P. tricornutum* suggest these microalgae use different photo-acclimation strategies.

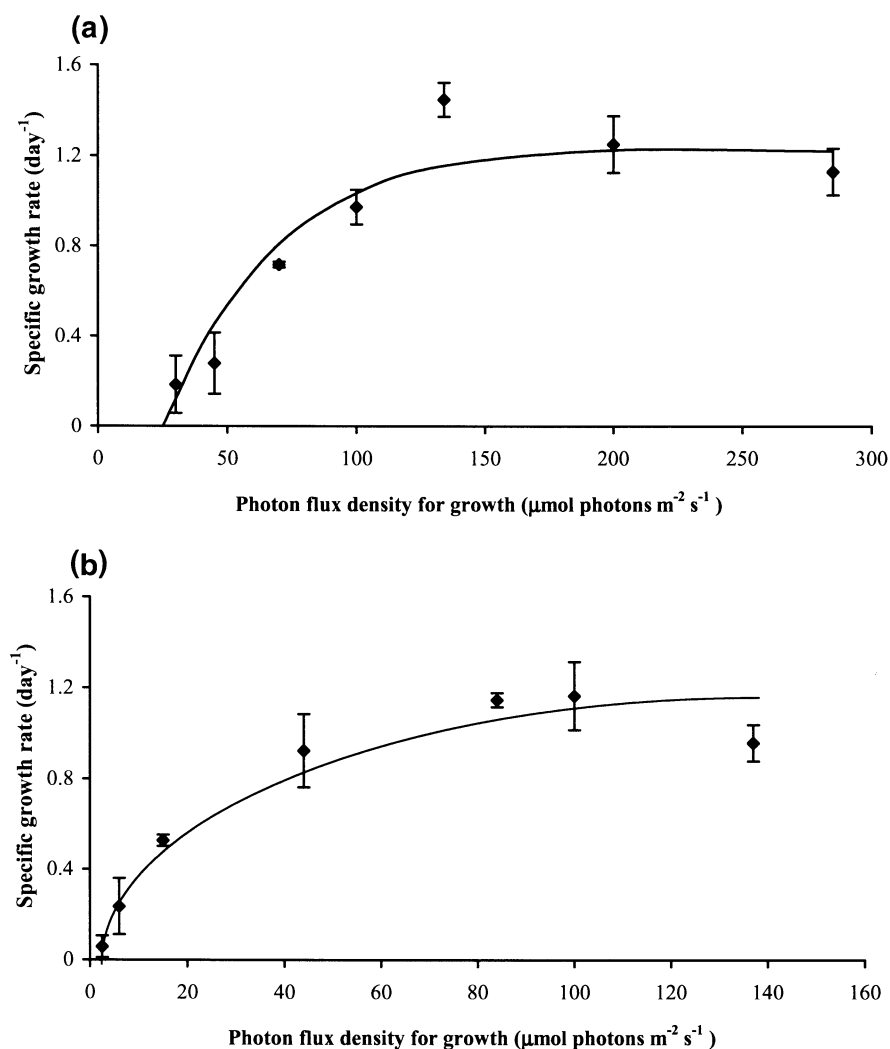


Figure 2. Growth rates of *D. tertiolecta* (a) and *P. tricornutum* (b) varied with photon flux between 0–300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Error bars (SE; $n \geq 8$) in some cases were smaller than symbols.

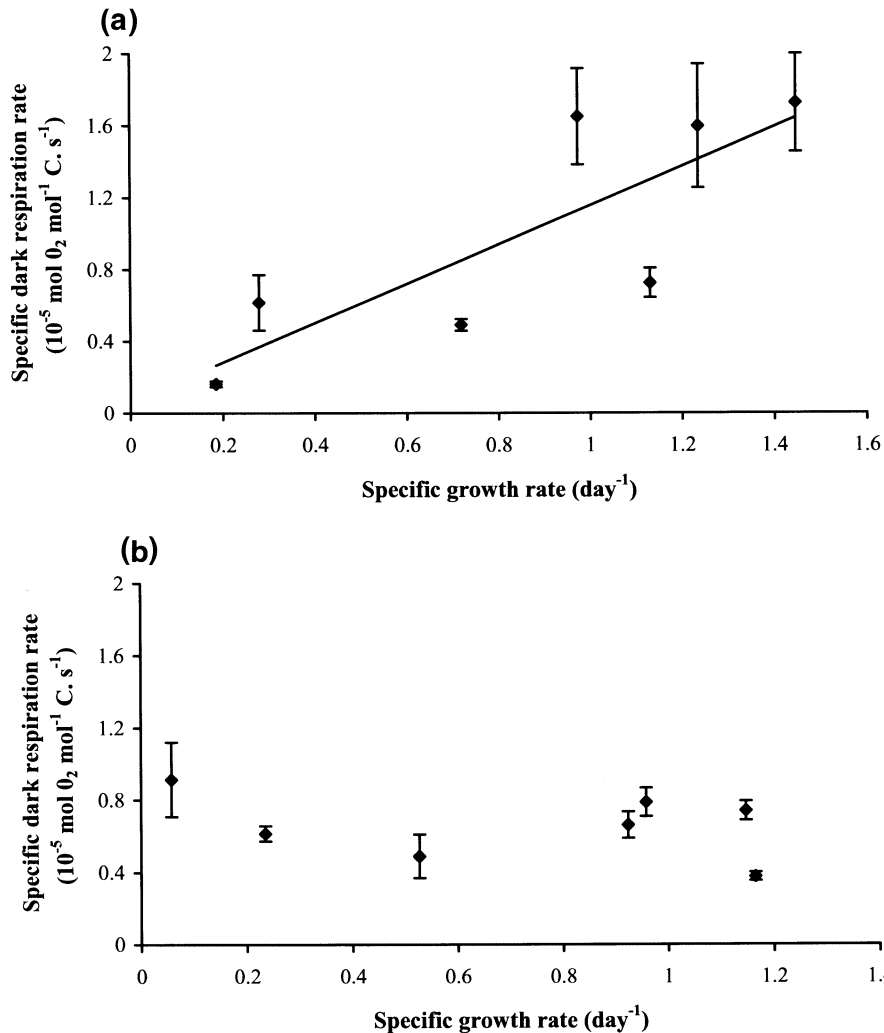


Figure 3. Specific dark respiration rates (R_d^c) increased with growth rate in *D. tertiolecta* (a). The linear regression describing this relationship is $y = 1.0x + 0.065$ ($r^2 = 0.66$). There was no significant change in R_d^c with growth rate ($P > 0.05$) in *P. tricornutum* (b). Error bars (SE; $n \geq 6$) in some cases are smaller than the symbols.

Rate constant of protein turnover

The $^3\text{H}_2\text{O}$ specific activity used in this study did not cause any detectable radiation stress or damage to cells. Growth rates in labelled and unlabelled cells were similar when a 20-fold range (1.85–37 kBq mL⁻¹) of $^3\text{H}_2\text{O}$ was used on both species grown at μ_{\min} and μ_{\max} (Quigg 2000). *Dunaliella tertiolecta* and *P. tricornutum* grown at μ_{\max} with 18.5 kBq mL⁻¹ $^3\text{H}_2\text{O}$ maintained a relatively constant $R_d : P_m$ ratio for the duration of the chase period, which was similar to that in control chambers and turbidostats (Table 2).

^3H completely labelled a small pool of rapidly turning over protein within the first 2 h of the pulse period (Fig. 1). Average incorporation rates were similar for *D. tertiolecta* ($18 \pm 2 \times 10^{-4}$ mol ^3H g protein⁻¹ h⁻¹; $n = 10$) and *P. tricornutum* ($17 \pm 1 \times 10^{-4}$ mol ^3H g protein⁻¹ h⁻¹; $n = 11$). This pool seemed to account for approximately 0.4% of the total protein in the cell (Quigg 2000). Thereafter, labelling showed a gradual increase suggesting slow entry of label into a larger protein pool (Fig. 1). This pool, however, did not appear to become fully labelled during the 24 h pulse, but presumably (ignoring the possibility of multiple pools

with different turnover times) was labelled sufficiently such that a long-term chase would still yield good estimates of the kinetics of long-term protein turnover.

Short-term protein turnover rates were measured in the first 10 h of the chase (Fig. 5a) whereas long-term protein turnover rates were measured over a 100 h chase (Fig. 5b). Rate constants for protein degradation at each photon flux were faster in *D. tertiolecta* than in *P. tricornutum* (Fig. 5a & b). Protein turnover rates increased with growth rate. The long-term protein turnover rates were found to be significantly different ($P < 0.05$) when μ_{\min} and μ_{\max} were compared. Short-term protein turnover rates however, did not vary significantly between treatments ($P > 0.05$).

Maintenance coefficient for protein turnover

The maintenance coefficient for protein turnover (mp') is the respiratory cost on a protein-N and time basis (mol O₂ mol⁻¹ N s⁻¹). The values of mp' for *D. tertiolecta* and *P. tricornutum* were estimated according to Eqn 1 (De Visser *et al.* 1992; Van der Werf *et al.* 1992; Bouma *et al.* 1994).

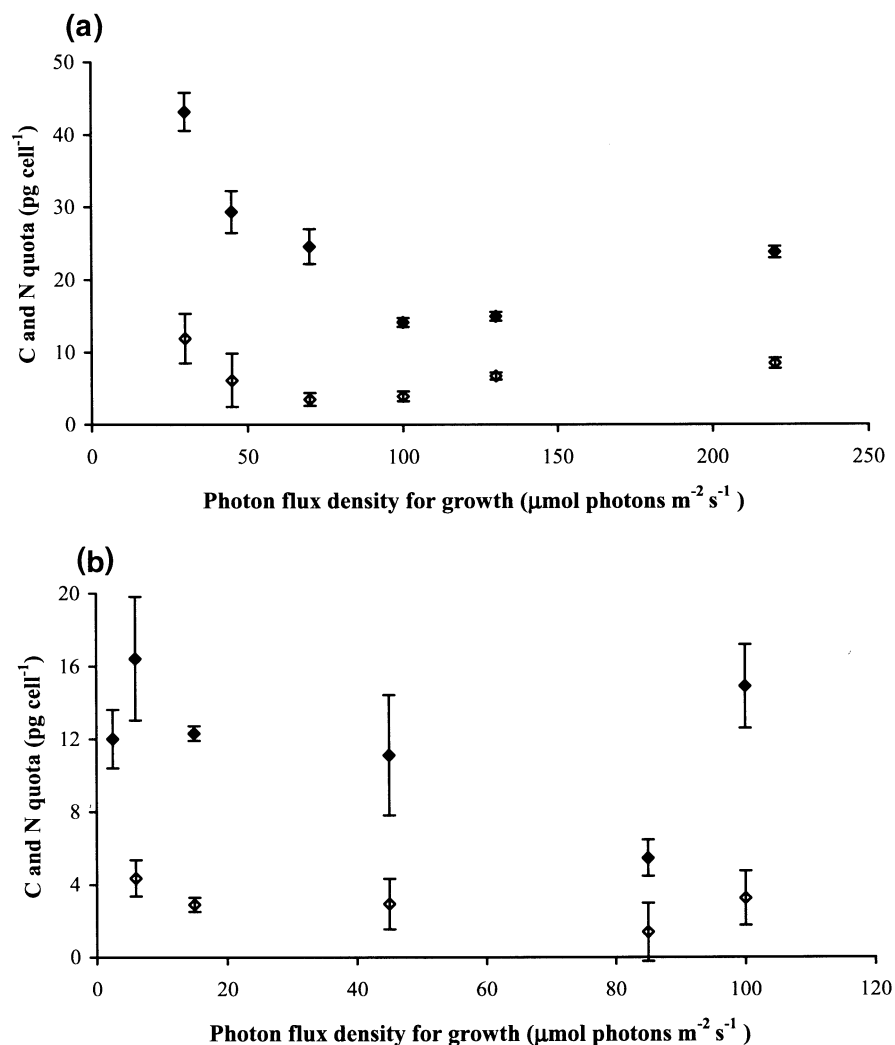


Figure 4. Cellular C (filled diamonds) and N (open diamonds) quotas decreased with photon flux for growth in *D. tertiolecta* (a) and *P. tricornutum* (b). Small increases were seen at photon fluxes > 200 μmol photon m⁻² s⁻¹ and > 100 μmol photon m⁻² s⁻¹ for *D. tertiolecta* (a) and *P. tricornutum* (b). Error bars represent SE ($n \geq 3$).

$$mp' = E_{sp} \times k_d \times k \quad (1)$$

Rate constants for protein degradation (k_d , d⁻¹) were taken from the zero growth rate intercept on Fig. 5b. The minimum theoretical specific ATP cost for protein turnover (E_{sp}) is 10.8 mol ATP mol peptide bond⁻¹ (Raven *et al.* 2000). A correction factor (k) was used to transform units of E_{sp} and k_d (De Visser *et al.* 1992). For direct comparisons of mp' with maintenance metabolic rates (below) and in the literature, we also then assumed that protein-N accounted for 85% of cellular-N (De Visser *et al.* 1992) and used the

Table 2. Average $R_d : P_m$ ratios (\pm SE) for *D. tertiolecta* and *P. tricornutum* grown at μ_{max} in turbidostats ($n \geq 12$) and chambers with and without ³H₂O ($n \geq 20$)

	$R_d : P_m$ ratio Turbidostats	$R_d : P_m$ ratio in chambers:	
		Control	Labelled
<i>D. tertiolecta</i>	0.230 (0.035)	0.259 (0.027)	0.299 (0.029)
<i>P. tricornutum</i>	0.228 (0.018)	0.267 (0.025)	0.271 (0.019)

protein pool sizes at I_{cg} (Table 1). On this basis, values for mp' were 6.37×10^{-6} mol O₂ mol⁻¹ C s⁻¹ (0.55 d⁻¹) and 0.84×10^{-6} mol O₂ mol⁻¹ C s⁻¹ (0.07 d⁻¹) for *D. tertiolecta* and *P. tricornutum*, respectively.

DISCUSSION

Photo-acclimation

Both laboratory experiments and field observations suggest that microalgae are capable of growth at extremely low photon fluxes, ≤ 1 μmol photon m⁻² s⁻¹ (Richardson, Beardall & Raven 1983; Geider *et al.* 1985, 1986; Falkowski & Raven 1997; Raven *et al.* 2000). Strategies employed are species-specific as seen in Figs 2–4 and Tables 1 and 2. *Phaeodactylum tricornutum* was able to sustain growth at photon fluxes an order of magnitude lower than *D. tertiolecta* (Fig. 2a & b), which involved lowered rates of R_d^C relative to those measured in *D. tertiolecta* (Fig. 3a & b; Table 1). Geider *et al.* (1985) have previously reported that R_d^C did not change significantly as a function of the photon flux for growth in *P. tricornutum*, with similarly elevated R_d^C

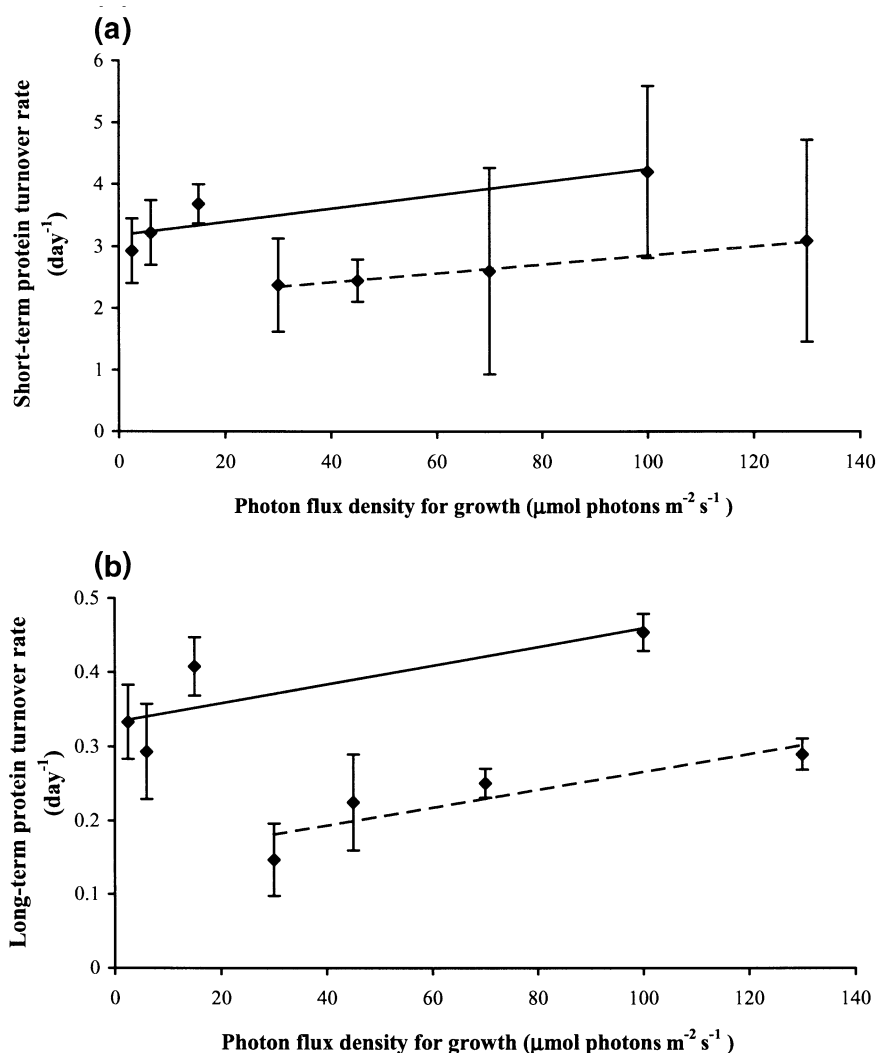


Figure 5. Short-term (a) and long-term (b) rate constants for protein turnover in *D. tertiolecta* (dashed line) and *P. tricornutum* (solid line) were plotted against the photon flux for growth. Mean rates are presented with SE ($n > 3$). Linear regressions describing this relationship are: *D. tertiolecta*: $y = 0.007x + 2.12$, $r^2 = 0.99$ (a), $y = 0.001x + 0.17$, $r^2 = 0.78$ (b); *P. tricornutum*: $y = 0.011x + 3.18$, $r^2 = 0.79$ (a), $y = 0.001x + 0.32$, $r^2 = 0.66$ (b).

at low photon fluxes for growth (Fig. 3b). We suggest that higher than predicted R_d^C may provide this alga with respiratory-derived ATP and reductant for continued growth in the virtual absence of photosynthesis, and hence be an important factor allowing this alga to successfully exploit variable environments.

An increase in R_d^C and P_m^C with photon flux for growth is considered the typical response for microalgae (Richardson *et al.* 1983; Langdon 1988; Geider & Osborne 1989) and higher plants (Lambers *et al.* 1998). This was observed for *D. tertiolecta* up to approximately $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, after which there was no real change in R_d^C (Fig. 3a) but a small decline in P_m^C (Table 1), suggesting that *D. tertiolecta* may have been photo-inhibited at these higher photon fluxes. Close to I_{cg} , C and N quotas rose significantly in *D. tertiolecta* (Fig. 4a). Cells were seen to accumulate starch (Quigg 2000) and increase the size of their protein pool (Table 1). Despite these physiological strategies, *D. tertiolecta* was not able to grow at photon fluxes as low as those exploited by *P. tricornutum*.

Protein turnover in eukaryotic microalgae

We still do not have a clear understanding of the *in vivo* rates, mechanisms and energy costs of biosynthesis and biodegradation of proteins and regulation of enzyme activity in microalgae. Long-term rate constants of protein turnover in *D. tertiolecta* and *P. tricornutum* ranged from 0.14 to 0.29 d^{-1} and 0.29 – 0.45 d^{-1} , respectively. These are comparable with rates of protein turnover in *Chlorella fusca* var. *vacuolata* and higher plants (Table 3). Protein turnover rates in leaves and roots are species-specific, and depend on both the growth rate and environmental conditions. Richards & Thurston (1980a, b) found that protein turnover rates in *C. vacuolata* decreased by 30% upon nitrogen starvation. Similar decreases in the rate of protein turnover have been measured in higher plants (Table 3). Therefore, as the growth rate decreases, so does the overall rate of protein turnover in bacteria, microalgae and higher plants.

Our estimates of protein turnover assumed synthesis and degradation occurred simultaneously. As such, we did not

Table 3. Reported measurements of the protein turnover rate (d^{-1}) and of the respiratory energy demand (%) associated with protein turnover in higher plants

Species	Characteristics	Rate of protein turnover (d^{-1})	% of maintenance respiration ascribed to protein turnover	Reference
Higher plants	Leaves	0.12	50–60%	Penning de Vries (1975) ^a
<i>Lemna minor</i>	Leaves	0.09 non-stressed 0.34 stressed	nd	Humphrey & Davies (1976)
<i>Chlorella fusca</i> var. <i>vacuolata</i>	Chlorophyte	0.30–0.51 non-stressed	nd	Richards & Thurston (1980a, b)
<i>Zea mays</i>	Leaves	0.12	nd	Simpson, Cooke & Davies (1981)
<i>Hordeum vulgare</i>	Leaves, seedlings	0.09 non-stressed 0.15 stressed	nd	Dungey & Davies (1982)
Higher plants	Leaves	0.42	nd	Davies (1982) ^a
<i>Lolium perenne</i>	Leaves	0.28	27–36%	Barneix <i>et al.</i> (1988)
<i>Dactylis glomerata</i>	Roots	0.065	7%	van der Werf <i>et al.</i> (1992) ^b
<i>Phaseolus vulgaris</i>				
<i>Solanum tuberosum</i>				
<i>Lolium perenne</i>				
<i>Hordeum vulgare</i>	Leaves, full-grown	nd	30–60%	De Visser <i>et al.</i> (1992)
<i>Solanum tuberosum</i>	Leaves, growing	0.04–0.21	17–35%	Bouma <i>et al.</i> (1994)
<i>Phaseolus vulgaris</i>	Leaves, full-grown	0.04–0.09		
<i>Triticum aestivum</i>	Leaves	nd	34–37%	Zagdańska (1995)
<i>Dactylis glomerata</i>	Roots, fast growing	0.156	22–30%	Scheurwater <i>et al.</i> (2000) ^b
<i>Festuca ovina</i>	Roots, slow growing	0.116	22–30%	

^aPenning de Vries (1975), Davies (1982) are reviews.

^bvan der Werf *et al.* (1992) and Scheurwater *et al.* (2000) assumed 50% recycling of the ¹⁴C-labelled leucine in their experiments. nd, not done

expect to see a net change in the cellular protein concentrations. In line with this, the protein pool size essentially did not vary in response to the photon flux for growth in *D. tertiolecta* and *P. tricornutum* (with only one exception; Table 1). Protein pool sizes have been shown to remain relatively constant as a function of the growth irradiance in other eukaryotic microalgae: *Thalassiosira weissflogii* (Post, de Wit & Mur 1985), *Chaetoceros gracilis*, *Isochrysis* aff. *galbana*, *P. tricornutum* (Thompson, Guo & Harrison 1993) and *Leptocylindrus danicus* (Verity 1982). Although the size of the protein pool *per se* remained unchanged, the amounts of specific proteins and the types of proteins synthesized at the extreme ranges of photon fluxes for growth would have varied. For example, light-harvesting complex proteins are synthesized to a greater extent in low-light-grown cells, while the opposite occurs for Rubisco (Falkowski & Raven 1997).

Proportion of protein turnover related to maintenance costs

Protein turnover is considered to be the largest single contribution to maintenance costs in all living cells (Penning de Vries 1975; Raven & Beardall 1981; Geider *et al.* 1996; Lambers *et al.* 1998; Raven *et al.* 2000). In order to determine what proportion of respiration was associated with protein turnover, the specific maintenance respiration rate (R_m) is calculated. Langdon (1988) and Geider & Osborne

(1989) reviewed estimates of R_m calculated when light was the limiting factor for growth in 17 species of microalgae. These varied between 0.11 and 5.09×10^{-6} mol O₂ mol⁻¹ C s⁻¹ (i.e. 0.009–0.44 d⁻¹ assuming a respiratory quotient of unity). However, the question of which is the most appropriate method to calculate R_m remains contentious.

Extrapolation of the growth rate versus irradiance relationship (Fig. 2a & b) to zero growth as proposed by Van Liere & Mur 1979) yielded a specific maintenance coefficient of 0.33 d⁻¹ for *D. tertiolecta* and 0.27 d⁻¹ for *P. tricornutum*. The intercept of the linear portion of the relationship between R_d^C and growth rate has also been used to estimate R_m (Laws *et al.* 1985). We did this only for *D. tertiolecta* (Fig. 3a), and calculated a value for R_m of 0.056 mol O₂ mol⁻¹ C d⁻¹. The model used by Geider *et al.* (1985, 1986) and Langdon (1988) is the method we preferred to estimate R_m , as this considers a number of physiological attributes that constrain growth (Eqn 2),

$$R_m = [a^* \phi_m I_{cg} \Theta Z]/PQ \quad (2)$$

and hence the magnitude of R_m . The Chl *a*-specific absorption coefficients (a^*) were taken from the literature: 0.0052 m² mg⁻¹ Chl *a* for *D. tertiolecta* (Welschmeyer & Lorenzen 1981 in Langdon 1988) and 0.0051 m² mg⁻¹ Chl *a* for *P. tricornutum* (Geider *et al.* 1985). The maximum theoretical quantum yield of photosynthesis (ϕ_m) is 0.125 mol O₂ mol⁻¹ photon. The Chl *a* : C ratios (Θ ; Table 1) at I_{cg} were 0.086 and 0.056 mg Chl *a* mg⁻¹ C for *D. tertiolecta*

and *P. tricornutum*, respectively. Z is the atomic weight of carbon ($0.012 \text{ mg mol C}^{-1}$). Photosynthetic quotients (PQ) for algae have been shown to depend on the nitrogen source during growth (Laws 1991). As we used nitrate in the growth media, we used a value for PQ of $1.4 \text{ mol O}_2 \text{ mol}^{-1} \text{ CO}_2$. The R_m calculated for *D. tertiolecta* ($8.62 \times 10^{-6} \text{ s}^{-1}$; 0.87 d^{-1}) was greater than that of *P. tricornutum* ($0.73 \times 10^{-6} \text{ s}^{-1}$; 0.06 d^{-1}). The R_m for *P. tricornutum* was similar to that previously reported by Geider *et al.* (1985) for the same species. Both our mp' (Eqn 1) and R_m values (Eqn 2) were an order of magnitude greater for *D. tertiolecta* than for *P. tricornutum*. These results are consistent with the literature which proposes that species that are best adapted to growth at low light exhibit lower maintenance respiration rates (Langdon 1988), with diatoms typically exhibiting lower R_m values than flagellates (Falkowski & Raven 1997). Variations in the magnitude of R_m calculated using the various models most likely reflects the associated assumptions.

Using the R_m calculated from Eqn 2, we estimated the proportion of maintenance respiration used for protein turnover (Table 4). Protein turnover was proportionately a more energetically expensive process in *D. tertiolecta* (36%) than in *P. tricornutum* (30%). These values fall within the reported range of 7–60% for higher plants (Table 3). The trade-off for high metabolic activity (requiring high protein concentrations) as seen in *D. tertiolecta* is an associated increase in the cost of maintenance. Higher respiration rates as measured in *D. tertiolecta* contributed to higher I_{cg} values, which, in turn, increased the minimum irradiance at which this alga was able to maintain a positive carbon balance. Hence, the contention that any factor that increases protein concentration or turnover rate will increase the maintenance respiration in higher plants

(Penning de Vries 1975; Davies 1982; Vierstra 1993) is supported by the findings for these two species of eukaryotic microalgae.

As nothing is known about the relative contribution of the protein pools with high and low turnover rates, calculations presented here have been based on the lowest protein turnover rates measured. The phenomenon for the short-term protein turnover rates is not so easily explained by our current understanding of the processes that govern protein turnover and maintenance. However, by using our estimate for the rapidly turned-over pool size (0.4%), we predict that short-term protein turnover rates account for only approximately 1.7 and 1.2% of the total maintenance cost in *D. tertiolecta* and *P. tricornutum*, respectively. Hence, short-term protein turnover rates will not influence cellular energy budgets, even at very low photon fluxes.

Species-specific ranges of protein turnover rates add to the known list of differences in the photo-acclimation strategies used by *D. tertiolecta* and *P. tricornutum*. The overall higher protein turnover rates and two-fold range measured in *D. tertiolecta* would be important in dealing with the potentially damaging effects of higher photon fluxes, as well as increasing protein pool sizes when growing at extremely low photon fluxes. The lowered maintenance metabolic energy requirement of *P. tricornutum* for cellular housekeeping, is arguably the most important factor governing its ability to grow at very low photon fluxes, along with the higher than predicted R_d^C . The present investigation examined the impact of protein turnover as a constraint limiting to photolithotrophic growth at low light. The two other constraints proposed by Raven *et al.* (2000) – charge recombination in photosystem II and proton leakage and slippage – are the subject of future investigations.

Table 4. An estimate of the proportion of maintenance respiration used for protein turnover by *D. tertiolecta* and *P. tricornutum*. The rate constant for protein turnover was measured at the zero growth rate intercept in Fig. 5a and b. The magnitude of the maintenance metabolic rate was estimated according to Eqn 2 (Geider *et al.* 1985, 1986; Langdon 1988). An ATP demand of 10.8/peptide bond is based on Raven *et al.* (2000) and the mean molecular weight per amino acid (110 Da) is from De Visser *et al.* (1992). The equivalent O_2 consumption rate was calculated by Penning de Vries (1975)

Estimate of the maintenance respiration rate	<i>D. tertiolecta</i>	<i>P. tricornutum</i>		
$\text{mol O}_2 \text{ mol C}^{-1} \text{ s}^{-1}$ (From Eqn 2)	8.62×10^{-6}	0.73×10^{-6}		
Carbon content (mol C cell^{-1}) at I_{cg}	4.83×10^{12}	1.0×10^{12}		
$\text{mol O}_2 \text{ cell}^{-1} \text{ h}^{-1}$	6.40×10^{-15}	2.63×10^{-15}		

Respiration rates based on protein turnover	Long-term	Short-term	Long-term	Short-term
Rate constant for protein turnover at zero growth rate (h^{-1})	0.007	0.09	0.013	0.13
Rate of turnover ($\text{g protein cell}^{-1} \text{ h}^{-1}$)	1.16×10^{-13}	14.4×10^{-13}	0.40×10^{-13}	4.03×10^{-13}
ATP demand at 10.8/peptide bond and mean molecular weight/amino acid of 110 Da ($\text{mol ATP cell}^{-1} \text{ h}^{-1}$)	1.14×10^{-14}	13.96×10^{-13}	0.39×10^{-14}	3.95×10^{-14}
Equivalent O_2 consumption rate assuming $0.5 \text{ O}_2 \rightarrow \text{H}_2\text{O}$ with translocation of 10 H^+ /ATP ($\text{mol O}_2 \text{ h}^{-1} \text{ cell}^{-1}$)	2.29×10^{-15}	2.79×10^{-14}	0.79×10^{-15}	0.79×10^{-14}
Protein turnover as a percentage of maintenance respiration	36%	1.7% ^a	30%	1.2% ^a

^aShort-term protein turnover costs as a percentage of maintenance respiration are adjusted, based on the estimation that only 0.4% of the protein pool is turned over rapidly.

ACKNOWLEDGMENTS

A.Q. would sincerely like to thank John Raven for his continued support. Karen Kevekordes, Zoe Finkel and Oscar Schofield read and provided useful comments on earlier drafts of this manuscript. Andrew Johnston of the Department of Biological Sciences, Dundee University, Scotland, generously performed the CHN analyses. We would like to thank two anonymous reviewers for helpful comments.

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Received 21 July 2002; received in revised form 25 October 2002; accepted for publication 29 October 2002