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Light environment and pigment composition of *Megaceros pellucidus*

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Abstract. The hornwort *Megaceros pellucidus* occupies wet, heavily shaded sites. In four sites in the central North Island of New Zealand the maximum photon flux density (PFD) was always less than 10 µmol m^{-2} s^{-1}. The PFD varied weakly during the day depending exponentially on the PFD of full sunlight. Light reflected from surface water late in the day transiently doubled the mid-day maximum PFD. Sunflecks had little impact on the site PFD consistent with the exponential relationship between the PFDs measured at the site and canopy. Plants from low light conditions (0.2 µmol m^{-2} s^{-1}) had the same carotenoid content as plants from higher light conditions (6.9 µmol m^{-2} s^{-1}), but the chlorophyll content of high light plants was approximately twice that of low light plants. The chlorophyll *a*/*b* ratio was the same for plants from low and high light conditions. Spectra of acetone-extracts of *M. pellucidus* thallus from low light grown plants showed an absorbance band at about 340 nm that was not apparent in spectra of extracts from plants grown in high light conditions.
Key Words: Anthocerophyta, carotenoid, chlorophyll, light environment, *Megaceros pellucidus*.

Résumé. *Megaceros pellucidus* occupe les sites qui sont mouillé et profondément ombragé. Á quatre sites au centre de l'île du Nord de la Nouvelle-Zélande le densité maximale de flux des photons (PFD) etait toujours moins de 10 µmol m^{-2} s^{-1}. La variation diurne du PFD etait faible et dépendait exponentiellement de la lumière de plein soleil. En fin de l'apres midi, le PFD de la lumière réfléchie par l'eau de surface était transitoirement deux fois de celui du maximum au milieu de la journée. Les faisceaux lumineux pommelés n'affectaient pas de manière significative le PFD en conforme avec la dépendance exponentielle entre le PFD en la site et en le couvert forestier. Les plantes cultivées á fort niveau de lumière (6.9 µmol m^{-2} s^{-1}) avaient la même teneur caroténoïde, mais deux fois la teneur en chlorophylle des plantes cultivés á faible niveau de lumière (0.2 µmol m^{-2} s^{-1}). Le rapport chlorophylle *a*/*b* etait semblable pour les plantes cultivés á faible et fort niveau de lumière. Les spectres des extraits acétoniques du thalle de *M. pellucidus* cultivé á faible niveau de lumière avait un bande d'absorbance prés de 340 nm. Les spectres d'extraits des plantes cultivées á fort niveau de lumière n'avait pas cet bande.
Mots clés: Anthocérotes, caroténoïde, chlorophylle, lumière environnemental, *Megaceros pellucidus*.

Rezumat: *Megaceros pellucidus,* este reprezentant al briofitelor şi populeaza zonele umede şi umbroase. În patru puncte din Insula de Nord a Noii Zeelande valoarea maximă a densităţii fluxului de fotoni (PFD) a fost întotdeauna mai mică de 10 µmol m^{-2} s^{-1}. PFD a variat uşor în timpul zilei depinzând exponenţial de densitatea solară a fluxului de fotoni. Lumina reflectată de suprafaţa apei după-amiaza a dublat adesea valoarea maximă a densităţii fluxului de fotoni de la mijlocul zilei. Fasciculele luminoase fragmentate au avut un impact minor la locurile de măsurare a fluxului de fotoni şi nu au afectat relaţia exponenţială măsurată la nivelul locului de creştere a plantelor comparată cu cea de la nivelul coronamentului. Plantele crescute în lumină slabă (0.2 µmol m^{-2} s^{-1}) au avut acelaşi conţinut în carotenoizi ca şi plantele crescute în lumină puternică (6.9 µmol m^{-2} s^{-1}), dar conţinutul în clorofilă a plantelor crescute în lumina puternică a fost de aproximativ două ori mai mare decât a plantelor crescute în lumină slabă. Spectrul extractelor de talus de *M. pellucidus* în acetonă de la plantele crescute în lumină slabă au indicat o bandă de absorbţie la aproximativ 340 nm care nu a aparut în spectrul celor crescute în condiţii de lumină puternică.
Cuvinte cheie: Anthocerophyta, carotenoizi, clorofilă, lumina naturala, *Megaceros pellucidus.*

Introduction. Anthocerophyta (hornworts) are small, dark-green plants that have a thin, flattened prostrate and lobed thallus (Campbell 1984; Schuster 1984). Mucilage cavities in the thallus often contain colonies of the nitrogen fixing cyanobacterium,

Nostoc, in a symbiotic relationship (Rai et al 2000; Adams & Duggan 2008). The Anthocerotophyta are found in moist shaded environments: in forests, along rivers, on moist banks and in wet shaded pasture. All species appear to have adapted to tolerate low light levels. The greatest diversity among the Anthocerotophyta is found in wet tropical forests (Schuster 1984), but even in cool temperate regions species diversity remains high (Richards 1984; Schuster 1984).

Shade plants, such as those growing on the rainforest floor, require little light to saturate photosynthesis (Boardman 1977), consistent with the low photon flux density (PFD) to which they are exposed. While the PFD on the floor of a rainforest can be variable, it tends to be greater than 10 μmol m^{-2} s^{-1} on sunny days. For example, Lee (1987) reported PFDs of 1.5–44.7 μmol m^{-2} s^{-1} on the floor of rainforests in Central America, corresponding to full sunlight PFDs of 422–1911 μmol m^{-2} s^{-1}. McDonald and Norton (1992) reported that only a fraction of PFDs were less than 10 μmol m^{-2} s^{-1} on the floor of rainforests in the South Island of New Zealand when about half of full sunlight PFD measurements were less than 1000 μmol m^{-2} s^{-1}.

The chlorophyll (chl) content of shade plant chloroplasts is generally higher than that observed in sun plants, which grow in full sunlight, but the carotenoid (car) content need not differ between sun and shade plants (Demmig-Adams 1998; Matsubara et al 2009). The chl *a*/*b* ratio of bryophtyes (often reported to be 1–2 (Rao et al 1979; Martin 1980; Green & Snelgar 1982; Marschall & Proctor 2004)) tends to be lower than that of non-bryophtye shade species (often reported to be 2.5–3.5 (Demmig-Adams 1998; Matsubara et al 2009)). Bryophyte chl/car ratios have been reported to be 3–12.5 (g g^{-1}) (Marschall & Proctor 2004) compared with about 3–6 (g g^{-1}) for other plants, although sun and shade plants need not differ in carotenoid content (Demmig-Adams 1998; Rosevear et al 2001; Matsubara et al 2009). However, there are no reports of the relationship between growth PFD on the content of chl and car or the chl *a*/*b* or chl/car ratios of bryophytes, other than unsubstantiated remarks that very low chl *a*/*b* and high chl/car ratios are associated with low light habitats (Marschall & Proctor 2004).

While the Anthocerophyta tend to be adapted to low light levels the light environment of the sites they occupy has not yet been described. Similarly, the effect of the light environment on chl and car content of the Anthocerophyta has not been reported previously. Here we report on the light environment and the pigmentation of the hornwort *Megaceros pellucidus* growing in four locations in the central North Island of New Zealand.

Materials and Methods. Four sites in the central North Island of New Zealand (Fig. 1) were chosen to represent a range of environments in which *M. pellucidus* (Colenso) E. A. Hodgs. occurred naturally. Initially, the plants were identified on the basis of their location, morphology, spore colour and the absence of pyrenoids from the chloroplast (Campbell 1995). Later, their identity was confirmed by comparison with named specimens in the Anthocerophyte collection held in the Massey University Herbarium and was subsequently confirmed by the late Dame Dr E. O. Campbell. It should be noted that several previously distinct synonyms and basionyms of *Anthoceros arachnoideus* Steph., *A. granulatus* Colenso*, A. longispirus* (Carrington & Pearson) Steph.*, A. pellucidus* Colenso, *M. arachnoideus* (Steph.) Steph., *M. flagellaris* (Mitt.) Steph., *M. grandis* (Asngstr.) Steph., *M. longispirus* (Carrington & Pearson) Steph., *M. membanaceus* (Colenso) E. A. Hodgs. and *M. zotovii* Khanna. have been included in the species *Megaceros pellucidus* (Colenso) E. A. Hodgs. (Glenny 1998).

Where necessary, specimens were collected from the site, placed in a plastic bag with some site water and kept cool. The specimens were then washed prior to being grown on a pre-formed 35° slope composed of 50% water-soaked vermiculite and 50% site substrate (crushed limestone). A 3 mm layer of site water was maintained in the bottom of the container to ensure humid conditions. The containers were covered in Gladwrap® then stored in a cool room at 4°C and at a PFD of 2 μmoles m^{-2} s^{-1}. Once a month all specimens were irrigated with Hutner's combined solution (Hutner et al 1950).

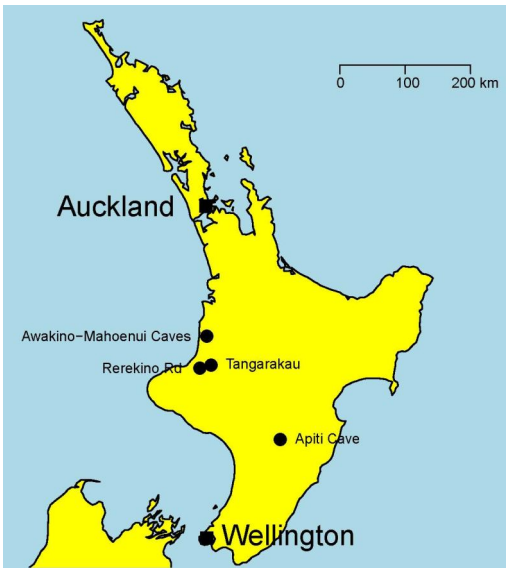

Figure 1. A map of the North Island of New Zealand showing the locations of the four sites (Apiti Cave, Rerekino, Awakino-Mahoenui Caves and Tangarakau) from which *M. pellucidus* was collected.

Within 24 hours of collection, chlorophyll (chl) was extracted from *M. pellucidus* thallus using dimethyl sulfoxide (DMSO) as described by Raemaekers (1987). Segments were cut from the youngest sections of the *M. pellucidus* thallus, flash frozen in liquid air and then dried with clean tissue. Weighed samples were placed in glass tubes and 5 mL of DMSO, sufficient to submerge each sample, was added. These were then incubated in a water bath at 65°C for 16 h, which Raemaekers (1987) recommended as giving optimal chl extraction with the minimal effect on the chlorophyll *a*/*b* ratio (chl *a*/*b*). If necessary, the tubes were stored in the dark at 4°C, which prevented any significant deterioration of chl *a*/*b* for up to 64 h (Raemaekers & Longwith 1987). The tubes were then centrifuged at 4500 rpm in a bench centrifuge and the supernatant was assayed using a Pye Unicam SP8-400 UV/VIS spectrophotometer with a 1.0 nm slit width. Total chl, chl *a* and chl *b* and total carotenoids (car) were determined using the absorbances at 663 nm (A_{663}), 645 nm (A_{645}) and 480 nm (A_{480}), and the expressions of Wellburn (1994)

$$\text{chl } a = 12.19A_{663} - 3.45A_{645}, \quad (1)$$

$$\text{chl } b = 21.99A_{645} - 5.32A_{663}, \quad (2)$$

$$\text{car} = \left(1000A_{480} - 2.14\,\text{chl } a - 70.16\,\text{chl } b\right)/220 \quad (3)$$

and it was assumed that total chl = chl *a* + chl *b*. The values obtained from (1-3) were not significantly different from the values obtained using the corresponding equations of Lichtenthaler (1987). All of these expressions give the pigment concentration (in mg L^{-1}) and were corrected for the mass of tissue.

Photon flux density was measured using a LI-250 quantum-radiometer fitted with an LI-190SA quantum sensor (LI-COR, Lincoln, USA), which is sensitive to photosynthetically active radiation between 400 nm and 700 nm. The manufacturer specified a measurement error of 0.01 µmoles m^{-2} s^{-1} at 25°C in the 0-199 µmoles m^{-2} s^{-1} range. When measuring the PFD, the sensor was held perpendicular to the major light source and reoriented until a maximum reading was obtained. This sensor position was then maintained over a 5 min period while the meter averaged the PFD.

Physical Description of Sites. Each of the sites (Fig. 1) had high surface water levels and heavy shading from natural land contours, forest top canopies and sub-canopies of ferns and other plants. Early-to-mid-Tertiary foraminiferal limestone substrate was found at all the sites (Hay 1967; Miller 1968) and a range of bryophytes were growing in the vicinity of each of the sampling sites. The water irrigating the *M. pellucidus* at all sites was slightly alkaline (pH 7.3 ± 0.1). Specimens were selected from random locations at

each site. The Apiti Cave and Rerekino sites were investigated in more detail than the other two sites.

Apiti Cave, known locally as the Apiti Glow Worm Cave, is situated off Limestone Road (39° 58′ 30″ S, 176° 0′ E) and consists of a cleft in a Tertiary limestone fault fracture. Water runoff flows down the sides of the cave and its surroundings, and a small river (Limestone Creek) runs through the cleft. While the site is referred to as a cave, it is actually an open-ended fault with a high arch. Similar smaller caves are situated upstream on the same fault line. Samples of *M. pellucidus* were taken at the mouth of and inside the cave at four different sites. The PFD was recorded at each sampling site, at the centre of the shaded river pond immediately in front of the cave and at an unshaded location on the road immediately above the cave. The unshaded, much higher PFD, was equivalent to that of the canopy top and was taken immediately above foliage, on the side of the road adjacent to the cave gully.

The Rerekino site is located on the southern side of the Rerekino spur approximately 200 m up on the right hand side from the end of the Rerekino Road (39° 01′ 30″ S, 174° 39′ E). Specimens of *M. pellucidus* were found growing in deep, heavily shaded recesses in foliage covered, water scoured channels situated in an old roadside cutting. In all cases the sampling sites had a constant water flow and were in deep shade from overhanging ferns and a tree canopy. The area was subjected to late afternoon (1600 h) sun was reflected by surrounding water and leaf surfaces.

The Awakino–Mahoenui Caves (AM Caves) are situated off North Taumatamaire Road (38° 36′ S, 174° 46′ E) where there are three caves, including Black's Cave from which samples were collected. The geology of this area comprises mudstone diversified with two up-thrusts of thin sections of early to mid-Tertiary foraminiferal limestone (Hay 1967). Each of the caves had a gravel and sandstone conglomerate floor. Water seepage from various parts of the wall forms pools in wall cavities and on the cave floor. The caves are shaded by ferns and trees, and the cave entrances are covered by *Elastostema rugosum* and aprons of severely dehydrated bryophytes cascading from the ceiling at the cave mouth.

The Tangarakau site (38° 59′ S, 174° 50′ E) was situated next to the northern abutments of the Tangarakau Gorge Road. High waterfalls cascade down these abutments to fall onto heavily shaded sheet greywacke with limestone inclusions. All of the *M. pellucidus* specimens were collected in the wet spray areas at the base of these falls. The sampling sites are heavily shaded by overhanging vegetation and, being on the southern side of the abutments, receive light early in the morning during the summer and late in the evening during late spring to early autumn. During the winter rock buttresses shelter the site completely from the sun except for a few hours in the middle of the day (from 1030-1100 h until 1400-1430 h).

Light Environment at the Sites. Despite the considerable range of PFDs measured at canopy level, the PFD in the *M. pellucidus* site was less than 10 μmol m^{-2} s^{-1} (Table 1). The light incident on the canopy was attenuated by the overhanging vegetation, so that the light incident on the cover immediately over the *M. pellucidus* site ranged from less than 10 μmol m^{-2} s^{-1} to more than 400 μmol m^{-2} s^{-1} (Table 1). The light available to the plants was 1.3–3.4 μmol m^{-2} s^{-1} (0.1–0.4% of the canopy PFD), but at particular times of day at the Apiti Cave and Rerekino sites light was reflected from the surface water onto the site, more than doubling the available light (Table 1).

The variation in the PFD during the course of the day was examined at the Apiti Cave site in autumn, winter and summer. The maximum PFD recorded at the canopy was 950–1100 μmol m^{-2} s^{-1}, depending on the season (Fig. 2A). However the variation in total PFD available at the canopy arose from the changing number of daylight hours. Numerical integration of the data in Fig. 2A provided estimates of the total PFD to be 43, 27 and 23 mol m^{-2} d^{-1} in summer, autumn and winter, respectively. The light at the site was less variable, reaching a maximum of about 2 μmol m^{-2} s^{-1} in the middle of the day, but on some days light reflected from surface water late in the day briefly reached 6.4 μmol m^{-2} s^{-1} (Fig. 2B). Numerical integration of the data in Fig. 2B provided estimates of

the total PFD to be 0.1, 0.04 and 0.03 mol m^{-2} d^{-1} in summer, autumn and winter, respectively.

Table 1

Photon flux density measurements for locations at each site. Each measurement was taken in the early afternoon and the errors represent ± SEM of between 10 and 30 separate measurements.

Site[a]	*Photon flux density (μmol m^{-2} s^{-1})*			
	canopy	over cover	reflection[b]	site[c]
Apiti Cave				
Summer	1081 ± 2	426 ± 8	6.6 ±0.1	1.3± 0.1
Autumn	924 ± 4	7.4 ± 0.2	–	1.5± 0.2
Winter	923 ± 23	270 ± 20	7.4 ±0.1	3.4± 0.7
average[d]	976 ± 25	235 ± 20	7.0 ± 0.8	2.1± 0.6
Rerekino	1555 ± 6	18 ± 6	6.1 ± 0.1	2.2± 0.4
AM Caves	1874 ± 40	39 ± 4	–	2.1± 0.4
Tangarakau	1076 ± 30	32 ± 4	–	3± 1

[a] Measurements were made at Apiti Caves at the summer and winter solstices and the autumn equinox.
[b] No reflections were observed at the AM Caves, at Tangarakau or at Apiti Cave during the autumn measurements.
[c] The values for the Apiti Cave are the average of 30 measurements (10 measurements at each of 3 sampling sites).
[d] The average refers to the mean of the summer, autumn and winter measurements obtained at Apiti Cave.

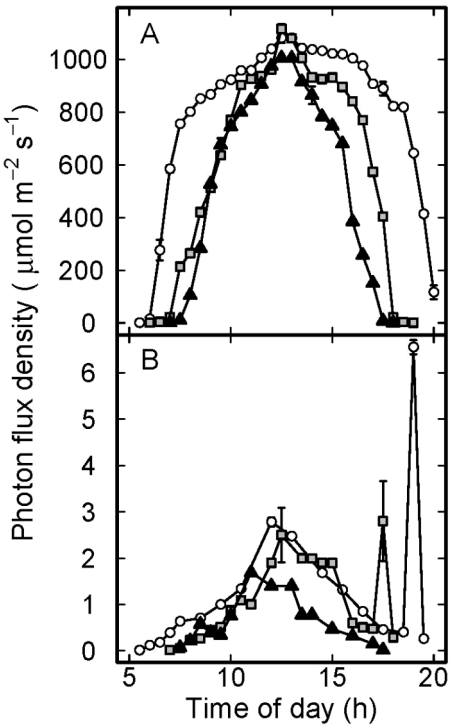

Figure 2. The PFD at canopy level (A) and at the sampling sites (B) within Apiti Cave over the course of a day in autumn (■, April), winter (▲, June) and summer (○, February). The points indicate the average (± SEM) of readings at four different sites within the cave. Most of the error bars are hidden by the symbols.

The PFD in the site was affected by the light available at the canopy (Fig. 2, A and B). The PFD in the site increased approximately exponentially with the PFD at the canopy (Fig. 3). Only those times at which light was transiently reflected onto the site from surface water (Fig. 2B, late afternoon in summer and autumn) did not conform to this relationship (Fig. 3).

The movement of the overhanging vegetation caused sunflecks that also caused

some variation in the site PFD. Measurements of the PFD (averaged over 5 min) were made over 155 minutes (between 1000 and 1225 h) during summer at a site that had a PFD of no more than 3 μmoles m^{-2} s^{-1} in the absence of additional light (sunflecks and reflected light) and a canopy PFD of about 1000 μmoles photons m^{-2} s^{-1}. The PFD above the vegetation covering the *M. pellucidus* site varied between 3 μmoles m^{-2} s^{-1} and 170 μmoles photons m^{-2} s^{-1} (Fig. 4). Despite this variation, the PFD at the site was no more than 3 μmoles m^{-2} s^{-1}, although the two measurements were weakly correlated and the distribution of PFD measured at each location was bimodal (Fig. 4).

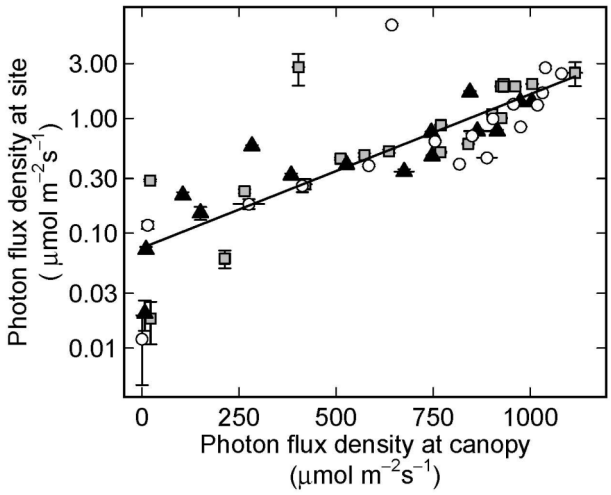

Figure 3. The relationship between PFDs at canopy level and at the sampling sites within Apiti Cave in autumn (■, April), winter (▲, June) and summer (○, February) shown in Fig. 2. The points indicate the average (± SEM) of readings at four different sites within the cave. Most of the error bars are hidden by the symbols. Note that the two points at the top of this plot represent light reflected off surface water. The line is the least squares fit to all of the data shown (slope = 0.0013 ± 0.0001, intercept = 0.07 ± 0.02, r = 0.82, p < 0.001).

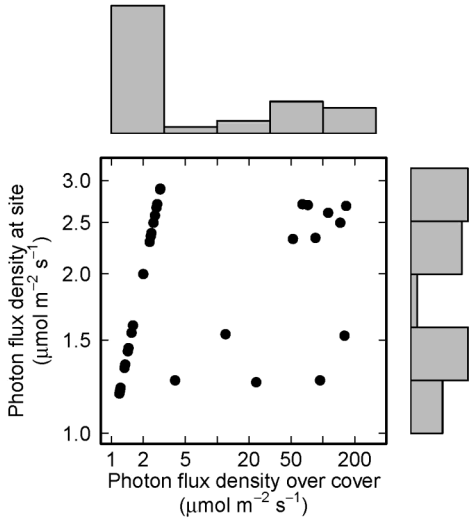

Figure 4. The relationship between the PFD at the site and above the cover immediately over the site during a period of vegetation motion causing sunflecks. The PFDs were averaged over 5 min intervals between 1000 h and 1225 h during summer at the Apiti Cave site. The marginal distributions illustrate the bimodal distribution of PFD at the site (right) and over the cover (top).

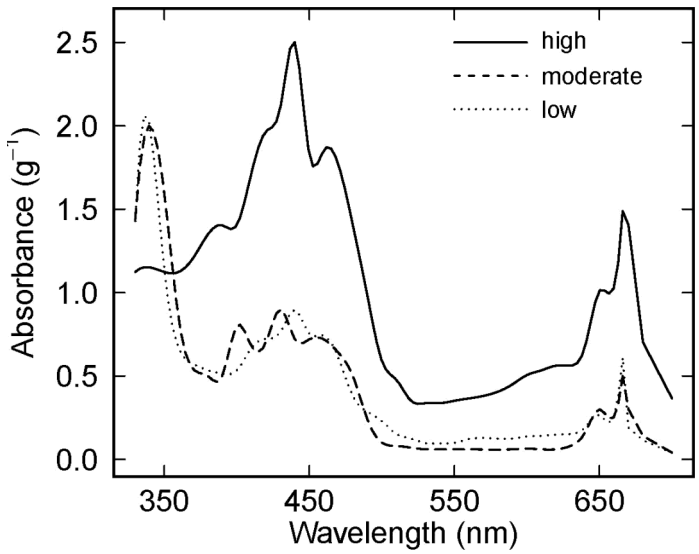

Figure 5. Absorbance spectra of 80% (*v*/*v*) acetone extracts of *M. pellucidus* grown in Apiti Cave in high (6.9 ± 0.3 µmoles photons m^{-2} s^{-1}), moderate (2.8 ± 0.1 µmoles photons m^{-2} s^{-1}) or low (0.2 ± 0.1 µmoles photons m^{-2} s^{-1}) light. The PFDs were measured at the Apiti site at 1400 h during April and absorbances have been corrected for differences in the wet weight of the tissue.

Efforts to characterise the quality of the light in the site were not successful. The very low PFD in the site inevitably meant that the contribution of any wavelength was small, which precluded reliable measurement.

The light harvesting pigments are conventionally extracted in a suitable solvent such as 80% (*v*/*v*) acetone or DMSO. However the pigment spectra are not significantly different in the two solvents ($p < 0.005$) (Raemaekers & Longwith 1987). Chlorophylls *a* and *b* absorb in the red (at about 660 nm) and in the blue (between about 400 and 460 nm), but do not absorb appreciably in the green (Fig. 5, high).

It was found that *M. pellucidus* grown in low light sites, exhibited an absorbance band with an apparent maximum at about 340 nm which was not apparent in pigment extracts from plants grown in higher light conditions (Fig. 5). Pigment extracts of *M. pellucidus*, grown in moderate or low light levels appeared to have very similar absorbance spectra (Fig. 5), but they both differed substantially from those of extracts from *M. pellucidus* grown in higher light sites (Fig. 5, high). This is apparent from the size of the ~660 nm absorbance bands (Fig. 5). While the ~660 nm bands were at the consistent wavelength, the peaks at shorter wavelength (400-500 nm) were not. In the high light plant spectrum these wavelengths were dominated by chl absorbance, but the moderate and low light spectra are less clear, which may reflect the contribution of car. Samples from the highest PFD conditions had significantly more chl than those from lower PFD situations (0.35 mg g^{-1} compared with 0.20 mg g^{-1}, Table 2).

Discussion. *Megaceros pellucidus* grew in extreme low light conditions (Table 1) which varied diurnally and seasonally, but were consistently less than about 10 µmol m^{-2} s^{-1} (Fig. 2B). The seasonal variation in the available light arose from changes in day length rather than changes in the maximum PFD (Fig. 2A). The basal maximum PFD of the site was approximately 4 µmol m^{-2} s^{-1}, but this was supplemented late in the afternoon by light reflected from surface water (Fig. 2B). Reflected light reached areas normally heavily shaded from any direct light and constantly changed because of the motion of the water surface. The site light available to *M. pellucidus* was largely determined by the light incident on the canopy (Fig. 3) and was little affected by sunflecks (Fig. 4).

Table 2. Pigmentation of *M. pellucidus* from low or high light sites. The values were calculated using (1-3) and the errors represent ± SE for at least 3 different measurements

Pigment	*low light*	*high light*
	0.2 µmol m^{-2} s^{-1}	6.9 µmol m^{-2} s^{-1}
chlorophyll *a* (mg g^{-1})	0.09 ±0.02	0.149 ±0.003
chlorophyll *b* (mg g^{-1})	0.11 ±0.01	0.20±0.01
total chlorophyll (mg g^{-1})	0.20 ±0.03	0.35±0.02
total carotenoids (mg g^{-1})	0.037 ±0.005	0.033 ±0.001
chlorophyll *a*/*b*	0.79 ±0.06	0.75±0.03
chlorophyll/carotenoids	5.6 ±0.1	10.6 ±0.1

The mean chl a/b and chl/car ratios were calculated from the values for each sample rather than from the means of the numerator and denominator.

The site light of *M. pellucidus* is only a fraction of the intensity of what is usully considered to be shade. For example, Lee (1987) reported PFDs of 1.5–44.7 µmol m^{-2} s^{-1} on the floor of rainforests in Central America, corresponding to full sunlight PFDs of 422–1911 µmol m^{-2} s^{-1}. The summer integrated PFD values reported here were similar to those of the crown canopy (40 moles m^{-2} day^{-1}) and leaf top of the sub- canopy (5 moles m^{-2} day^{-1}) in a tropical Australian rainforest (Chazdon et al 1996). McDonald and Norton (1992) reported that only a fraction of PFDs were less than 10 µmol m^{-2} s^{-1} on the floor of rainforests in the South Island of New Zealand when about half of full sunlight PFD measurements were less than 1000 µmol m^{-2} s^{-1}. Moreover, the integrated shade PFDs reported by McDonald and Norton (1992) were 0.2–7.7 mol m^{-2} d^{-1}, compared with 0.03–0.1 mol m^{-2} d^{-1} observed at Apiti Cave. If the floor of a rainforest is a shade environment, *M. pellucidus* occupies extreme shade sites.

We have not measured the photosynthetic light response of *M. pellucidus*, but PFDs of about 45–85 µmol m^{-2} s^{-1} saturated the photosynthetic apparatus of the liverworts *Marchantia polymorpha*, *Marchantia foliacea* and *Monoclea forsteri* (Mache & Loiseaux 1973; Green & Snelgar 1982), although the data of Marschall and Proctor (2004) indicate that higher PFDs (79–679 µmol m^{-2} s^{-1}) were required to saturate the photosynthesis of seventeen liverwort species, including *M. polymorpha*. It is likely that such apparent discrepancies arise from the environmental history of the plants rather than any physiologically significant difference (Rosevear et al 2001), and that the PFD required to saturate the photosynthesis of *M. pellucidus* would be even lower than 45–85 µmol m^{-2} s^{-1}. The remarkable growth of *M. pellucidus* in such low light is comparable to the ability of some other bryophytes to grow in complete darkness, given some nutrients (Servettaz 1912; Servettaz 1913; Robbins 1918).

The summer PFD in the *M. pellucidus* sites was almost twice those of the autumn and winter periods. However, sporophyte production, which requires expenditure of significant energy, occurred during the period of apparent minimal energy availability. This phenomenon is also observed with *Anthoceros laevis* and *Anthoceros punctatus*, which become fertile in short (8-12 h) days, and remain sterile in long (18 h) days (Benson-Evans 1964; Ridgway 1967).

By the time solar radiation reaches the forest floor, it is dramatically altered in quality as well as quantity (Boardman 1977; Gates 1980; Richards 1984; Pearcy 1989; Björn 1994; Kendrick & Kronenberg 1994; Niinemets et al 1999; Nobel 1999; Koller 2000). The low PFD at the sites investigated here precluded any reliable analysis of the light quality, but presumably the proportion of blue and red wavelengths would be reduced and that of far red (>680 nm) light would be increased by the overhanging vegetation (Smith & Morgan 1981; Smith 1986; Tang 1997).

Reflections from water surfaces (pools, rivers, wet leaves, wet rocks, water droplets) that were significant late in the day (Fig. 2B) are polarised (Chen & Rao 1968; Kraml 1994) and tend to be higher in far red and red wavelengths as a result of light

scattering (Olesen 1992). The polarisation of the light may be significant in the photosynthetic responses of *M. pellucidus* because it plays a role in inducing chloroplast movement (Senger 1984; Können 1985; Haupt & Hader 1994; Kagawa & Wada 1996; Yatsuhashi 1996; Dong et al 1998; Haupt 1999). The enrichment of red and far-red wavelengths at the *M. pellucidus* sites would be enhanced by the reflected light.

Water was abundant at all four sites. Bryophyte photosynthesis is highly sensitive to changes in tissue water content (Lee & Stewart 1971; Dilks & Proctor 1979), but droplets or films of water on the dorsal surface or trapped in trichomes can act as a lens, concentrating light up to 20 times (Brewer et al 1991). However, CO_2 diffuses through water about 10^4 times more slowly than it does through air (at 20°C) and even more slowly at lower temperature (Lawlor 1993), which might be expected to limit photosynthesis in higher light conditions.

The colour of *M. pellucidus* was also typical of bryophytes growing in extremely low light situations (Richards 1932), which are very dark green unless viewed from above in which case the plant appears to be an intense jade green. The chl content of *M. pellucidus* adapted to low light (0.2 µmol m^{-2} s^{-1}) was about 60% of that of high light (6.9 µmol m^{-2} s^{-1}) adapted plants (Table 2). The absorbance spectra of the pigment extracts are consistent with this, in that plants from high light sites had more chlorophyll than those from moderate and dark sites, but the spectra of pigment extracts of plants from the two low light conditions have an absorbance band at about 340 nm which is not apparent in the pigment extract from high light grown plants (Fig. 5). It is not clear whether this was a novel pigment or whether it was present in all the tissue samples and simply became more apparent as the chl content decreased. There are at least three potential explanations. First, this band might represent a modified chlorophyll. For example, bacteriochlorophyll *a* has an absorbance band with a peak at about 357 nm (70 nm lower than the 428 nm band of chl *a*), but it has another at about 768 nm (Sauer 1975), which was outside the range of the data collected here. Second, while the Anthocerophyta lack anthocyanins (Schuster 1984), phenylpropanoids (precursors of pterins and flavonoids) are abundant within the phylum (Becker 1994) and might be the unidentified chromophore giving rise to this absorbance band. Third, since there is a mutualistic association between *Nostoc* sp. and *M. pellucidus*, the polysaccharide-linked mycosporine E335 found in *N. commune* (Cockell & Knowland 1999), which has an absorbance maximum at 337 nm, might account for this absorbance band. Fourth, the band might represent the *cis*-peak of the carotenoids extracted from the plant (Zechmeister 1944). The chl content of shade plants tends to be higher than in those receiving full sunlight (Böhning & Burnside 1956; Anderson et al 1973; Tanaka & Melis 1997; Barbour et al 2000), but *Hydrilla verticillata* grown at a PFD of 6 µmol m^{-2} s^{-1} had less chl than when grown at 30 µmol m^{-2} s^{-1} (Bowes et al 1977) and the deeper it was grown the lower the chl content (Van et al 1977). But given the extremely low light circumstances in the *M. pellucidus* sites the pigmentation could be expected to be unusual.

The chl *a*/*b* ratio was about 0.75 for both shade and high light plants (Table 2). This extremely low value is consistent with the general reduction in the ratio in shade plants compared to sun plants (Boardman 1977; Lichtenthaler et al 1982; Björn 1994; Kendrick & Kronenberg 1994; Chazdon et al 1996), but even the high light regime was less than 5 µmoles m^{-2} s^{-1} (Table 1 and Fig. 2B), which is considerably lower than the PFD in which most understorey plants are found (Lee 1987; McDonald & Norton 1992; Chazdon et al 1996). The protonema of *Ceratodon purpureus* and the thallus of *M. polymorpha* have been reported to have a chl *a*/*b* ratio of only slightly greater than 2 (Aro 1982). However, unlike *M. pellucidus*, *M. polymorpha* is high light tolerant as long as adequate moisture is available, and *C. purpureus* is essentially a high light plant. The extremely low chl *a*/*b* ratios reported here (Table 2) are only slightly smaller than those of the alga *Mantoniella squamosa* grown at a PFD of only 15 µmol m^{-2} s^{-1} (Wilhelm et al 1997). Similar chl *a*/*b* ratios have also been reported for a chl *b*-less chlorophyllide *a* oxidase mutant of *Arabidopsis thaliana* after transformation with the homologous gene

from *Prochlirothrix hollandica* (Hirashima et al 2006). This observation prompts the suggestion that the supply of chl *b* might be a significant factor in determining the chl *a*/*b* ratio. Irrespective of this, the low chl *a*/*b* ratio observed here is, presumably, related to the extremely low light site in which *M. pellucidus* grows (Table 1 and Fig. 2B).

Conclusions. *Megaceros pellucidus* occupies wet sites in which the PFD is even lower than that supporting the growth of normal shade plants. The chl content of plants adapted to high light (6.9 µmol m^{-2} s^{-1}) was greater than that of plants adapted to low light (0.2 µmol m^{-2} s^{-1}), although their car content did not differ. The chl *a*/*b* ratio was even lower than that reported for other bryophytes which may be related to the extremely low light site occupied by *M. pellucidus*. How *M. pellucidus* copes with the light environment of its site remains to be explored.

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