

Water relations and carbon gain are closely related to cushion size in the moss *Grimmia pulvinata*

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SUMMARY

The present study of structural and physiological changes during the development of the cushion moss, *Grimmia pulvinata*, quantifies the size-dependence of various parameters of water relations such as changes in surface:volume ratio (S/V) or water loss rates, and also measures net CO_2 gas exchange in the light and the dark. Larger cushions had lower S/V values than smaller ones and featured lower rates of area-based evapotranspiration, owing to higher boundary-layer resistance, but did not differ in relative water storage capacity (expressed as a percentage of d. wt). In combination, this leads to considerably longer hydration periods in larger cushions. By contrast, CO_2 gas-exchange parameters were negatively correlated with size: larger cushions showed significantly lower (mass-based) rates of net photosynthesis and dark respiration. Using these data, we estimated carbon budgets during a drying cycle as a function of cushion size. When including alternations of dark and light periods, the relationship proved to be rather complicated. Depending on the time of hydration, net carbon budgets not only varied quantitatively with size but sometimes took on both positive and negative values depending on cushion size. We conclude that neglecting plant size can lead to unrepeatable or even misleading results in comparative ecophysiological studies, and therefore urge for adequate attention to be paid to size in these studies.

Key words: allometry, bryophytes, carbon budget, ecophysiology, net photosynthesis, water relations.

INTRODUCTION

Under natural conditions, poikilohydric organisms such as mosses and liverworts are generally subject to intermittent desiccation. Because carbon (C) gain is only possible when wet, growth is generally assumed to be directly proportional to the total length of the wet state (Proctor, 1972). Consequently, species differences in life form (Bates, 1998; 'growth form' *sensu* Gimingham & Robertson, 1950) are frequently interpreted as important adaptations to particular environmental conditions because of the obvious differences in respect of the storage of water or the prevention of rapid water loss, re-

spectively (Schofield, 1981; Proctor & Smith, 1995). Much less attention has been paid to intraspecific differences in form. One source of such variation between members of a given species is growth (which leads to difference in size and, possibly, form), but until now there has been no quantitative study of the effect of size on plant C gain for any moss or liverwort species (but compare studies with lichens (e.g. Larson, 1984; Gauslaa & Solhaug, 1998)).

Gimingham & Robertson (1950) distinguished five main 'growth forms' in terrestrial bryophytes, namely cushions, turves, mats, dendroids and wefts. Of these, the cushion form seemed most appropriate for an investigation of the influence of size on morphological and ecophysiological traits because of its rather simple geometry. In comparison with other 'growth forms', the evapotranspiring surface is reduced relative to the stored volume of water. The S/V is minimized in a hemisphere, which can therefore be used as a null model. Because of its large size range we chose *Grimmia pulvinata* for the

*Author for correspondence (tel +49 931 888 6223; fax +49 931 888 6235; e-mail zotz@botanik.uni-wuerzburg.de). Abbreviations: Δw , vapour pressure deficit; DM, cushion d. wt; E_{max} , maximum rate of evaporative water loss; NP, net photosynthesis; PFD, photon flux density; R_{D} , dark respiration; S , upper surface area of a cushion; S/V , surface:volume ratio; t_{50} , time required to lose 50% of WC_{max} ; V , cushion volume; WC, plant water content as a percentage of DM; WC_{min} , cushion WC when air-dry.

present study. The specific objectives were the quantification of the size dependence of water relations parameters such as the water-holding capacity and water loss rates, changes in S/V values, and an analysis of the resulting differences in activity periods. Furthermore, we studied net CO_2 exchanges as a function of size, both in the light and in the dark. Using these data for simple model calculations, we estimated the influence of cushion size on overall C gain under different light and dark cycles.

MATERIALS AND METHODS

Plant material

Grimmia pulvinata (Hedw) Sm. (Grimmiaceae) is a common epilithic (rarely epiphytic) cushion moss with almost worldwide distribution (Frahm & Frey, 1992). All samples in the present study were collected from limestone walls in the Würzburg Botanical Garden (Germany). Subsequently, they were cleaned carefully under a dissecting microscope; tiny pebbles or animals were removed. Finally, after an experiment, samples were dried for 4 d at 70°C, and their dry weight (DM) was determined with an analytical balance (SBA 21; Scaltec, Heiligenstadt, Germany; accuracy 0.1 mg).

Determination of cushion surfaces and volumes

Disregarding the fractal nature of cushion surface area, we treated a cushion as a smooth simple object (Proctor, 1980), which allowed us to estimate the upper surface area (S), the projected area (P) and the volume (V) of each cushion. On a total of 27 specimens of *G. pulvinata* (diameter 11–57 mm; DM 0.02–3.6 g) we measured the height of a cushion with a Leitz Micro-Manipulator (Leitz, Wetzlar, Germany) and a dissecting microscope. A probe 0.1 mm in diameter was lowered perpendicularly onto the specimen until it touched any part of the cushion except the dead hair points. Height could be measured reproducibly to the closest 0.5 mm. Starting approximately in the middle of a specimen and moving to the periphery, measurements were repeated every 0.5–4 mm. The number of repetitions depended on the size of the specimen and the surface structure. From the same starting point, similar measurements from centre to periphery were conducted at azimuth angles of 90°, 180° and 270°. Even for the smallest cushions (basal diameter 11 mm), this procedure yielded at least 16 measurements of height in relation to the distance to the centre. Each cushion was treated as a solid body consisting of four frustums, i.e. parts of a cone between two parallel cutting planes (F_1 – F_4). V and S of each frustum were estimated four times separately by using the four data sets from centre to periphery. The final estimates of the total V and S for each cushion were

determined by summing the average values of the four frustums:

$$S = \sum_{F=1}^4 \sqrt{(r_2 - r_1)^2 + \Delta h^2} * \pi * (r_1 + r_2) \quad \text{Eqn 1}$$

$$V = \sum_{F=1}^4 \Delta h \pi / 3 (r_2^2 + r_1 * r_2 + r_1^2) \quad \text{Eqn 2}$$

(r_1 and r_2 , the upper and lower radii of the frustum; Δh , the distance between the upper and lower surface of the frustum (in the uppermost frustum, $r_1 = 0$).

Measurements were taken in the laboratory at an ambient humidity of 50–60% on both air-dried and water-saturated cushions (water adhering superficially was removed by shaking the samples). The water content (WC) in the first case averaged 22% DM, in the latter 730% DM.

The deviation of the estimated S/V from minimal values of S/V (i.e. those of a hemisphere) were calculated as follows: first we calculated the surface area of a hemispherical body with a given volume V (for each sample) and then divided the surface area of a hemisphere (= S_{\min}) by V . The comparison of S/V and S_{\min}/V yielded the desired values (as percentages).

CO₂ gas exchange measurements

CO_2 exchange as a function of plant WC (as a percentage of DM) and cushion size was studied in the laboratory with 27 additional cushions (diameters 8–38 mm; DM 0.02–1.1 g) using a ‘mini-cuvette system’ (Walz, Effeltrich, Germany). Artificial, saturating illumination provided a PFD of *c.* 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$; the temperature inside the cuvette was 21°C. All CO_2 measurements were made with BINOS infrared gas analysers (Rosemount, Hanau, Germany) operating in differential mode. An additional BINOS in absolute mode was used to determine the CO_2 concentration of the air flowing through the system (380–420 $\mu\text{l l}^{-1}$). Differences in the CO_2 concentration could be measured to within 0.1 ppm. In brief, after an initial 12 h of hydration at low light (*c.* 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, WC > 500% DM) samples were transferred to *c.* 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD for *c.* 10 min to ensure photosynthetic induction. Samples were submerged in distilled water for *c.* 1 min and taken out; superficially adherent water was then removed by gentle shaking. Samples were put in wire-mesh baskets (3.8 cm in diameter, 1–1.5 cm in height, mesh width 1.6 mm) and then placed into the gas-exchange cuvette. Net photosynthesis (NP) was measured as soon as equilibrium had been reached. Then samples were removed and weighed immediately on an electronic balance (PM 460; Mettler, Giessen, Germany; accuracy 1 mg). After a few minutes, during which the samples were left to dry in the laboratory, they were enclosed in the cuvette again. This procedure resulted in a stepwise water loss and was continued until no more

changes in the relative water content occurred. In a similar manner, rates of dark respiration (R_D) were determined for 14 samples at 16°C.

Determination of rates of water loss

The rate of water loss was determined for 11 cushions of various sizes (diameter 5–40 mm; DM 0.0026–1.3 g) in a small climate chamber (PFD *c.* 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, wind speed *c.* 1.4 m s^{-1}). All cushions were initially saturated with distilled water; water adhering superficially was removed by gentle shaking. The samples were then put on pieces of plastic (4 cm \times 4 cm) and placed in the chamber together. At *c.* 20 min intervals, samples were weighed to the nearest 0.1 mg. An experiment lasted until all water had been lost and at least three determinations yielded the same weight. We repeated this desiccation cycle five times at various temperatures and relative air humidities to create a range of different conditions (air temperatures of 4, 10, 16, 21 and 27°C), representing vapour pressure deficits (Δw) of, respectively, 0.4, 0.45, 0.5, 0.8 and 2.2 kPa.

Data analysis

Data analysis was performed with STATISTICA software (STATISTICA 5.1; StatSoft Inc., Tulsa, OK, USA). Owing to the skewness of the distribution of cushion size, $\log_{10}\text{DM}$ was used for linear regression analysis (Sokal & Rohlf, 1995). Unless stated otherwise, means are shown with ± 1 SD as a measure of variance.

RESULTS

Morphometric changes with size

The cushions in this study encompassed a wide range of sizes, namely from 11–57 mm diameter. The nonsignificant correlation of specific mass (g mm^{-3}) with DM (g) of a cushion indicated that the density of a moss did not change with size ($r = 0.14$, $P = 0.26$, $n = 27$). Several parameters such as the surface area, the projected area and the volume of a cushion were highly predictable from moss DM (Table 1).

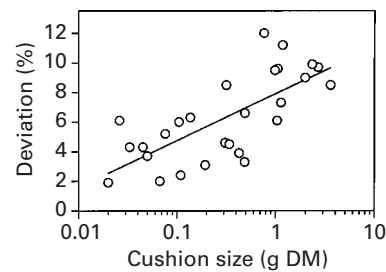


Fig. 1. Deviation of the surface:volume ratio of wet *Grimmia pulvinata* cushions from that of a hemisphere with similar volume, as a function of size (dry weight, DM). The 27 cushions ranged from 0.02 to 3.6 g DM. The regression line is: deviation (%) = 7.9 + 3.2 log DM; $r^2 = 0.52$, $P < 0.001$.

An important parameter in the context of the water relations of a poikilohydric organism is the ratio of S to V . S/V values decreased significantly with cushion size (i.e. DM) of both wet and dry cushions ($r \geq -0.96$, $P < 0.001$, $n = 27$). Excluding a sphere (because this shape is not biologically meaningful in the context of this study), the lowest S/V is achieved by a hemisphere. We computed these theoretical S/V values for all specimens and compared them with the actual S/V values. Small cushions deviated very little from the minimal S/V (as little as 2%), whereas larger specimens increasingly diverged from a hemispherical shape (Fig. 1). In other words, smaller cushions are almost hemispherical and larger individuals are increasingly flattened. To illustrate the effect of these changes in shape, we compared the measured surface area of a small cushion (0.02 g DM; volume 266 mm^3 ; surface 162 mm^2) with the calculated surface area of the same moss, but assuming the shape of the largest moss of the study (3.6 g DM): such a moss was expected to have a surface area of 177 mm^2 , i.e. 10% more. Water-holding capacity (i.e. the maximum WC) did not change with size ($P = 0.22$): it averaged $730 \pm 166\%$ DM.

Evaporation rates and size

One consequence of the described size-related changes in S/V are differences in the activity period of a moss after full initial hydration (time with

Table 1. Relationships between various morphological characteristics of *Grimmia pulvinata* cushions and moss dry weight (DM)

Parameter	Units	Condition	Regression equation	r^2
H (maximum cushion height)	mm	wet/dry	$H = 11.2 + 4.4 \log \text{DM}$	0.91
P (projected area)	mm^2	wet	$P = 193 + 1038\text{DM} + 130\text{DM}^2$	0.92
S (surface area)	mm^2	air-dry	$S = 2662\text{DM}/(1 + 0.96\text{DM})$	0.96
S (surface area)	mm^2	wet	$S = 3521\text{DM}/(1 + 0.82\text{DM})$	0.95
V (volume)	mm^3	air-dry	$V = 79 + 6815\text{DM} - 592\text{DM}^2$	0.94
V (volume)	mm^3	wet	$V = 647 + 8337\text{DM} + 555\text{DM}^2$	0.93

Shown are the best-fit nonlinear regression equations and the correlation coefficients both for air-dry and fully saturated (= wet) cushions ($n = 27$). The equations are valid for a DM of 0.02–3.6 g.

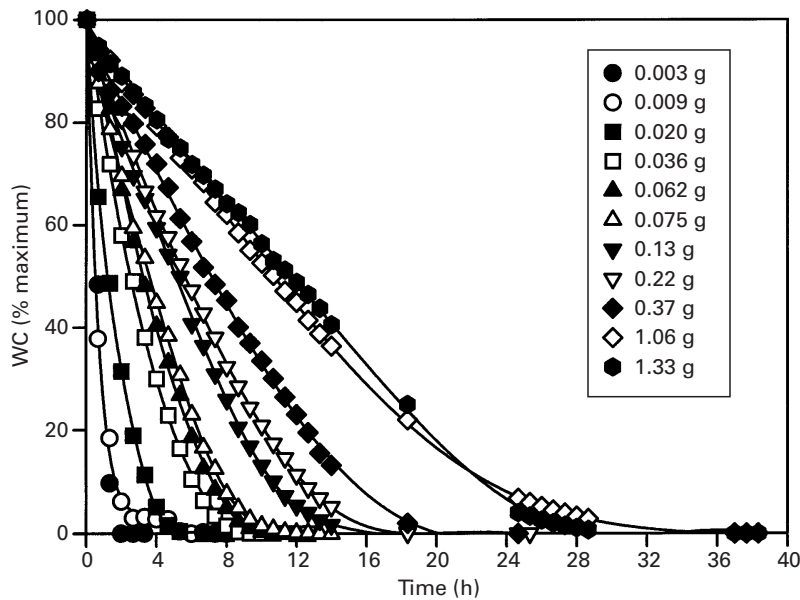


Fig. 2. Decline in cushion water content (WC, as a percentage of maximum water content) during a drying cycle in a climate chamber (temperature 16°C, relative air humidity 70%, Δw 0.5 kPa, wind speed 1.4 m s⁻¹) as a function of cushion dry weight. The lines are fifth-degree polynomial regressions ($r^2 \geq 0.90$).

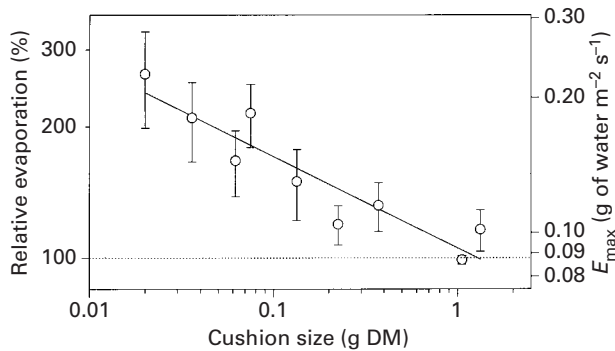


Fig. 3. Relationship between evaporation rate and cushion size (dry weight, DM). For each of nine samples during five drying cycles (similar to that shown in Fig. 2, but at five different temperatures) the initial constant rates of water loss (E_{\max}) were determined and expressed as percentages of the lowest evaporation rate, found in the cushion with 1.06 g DM (set to 100%, 85.7 mg m⁻² s⁻¹). Results are means \pm SD, $n = 5$. The regression line is: $\log E_{\max} = -1.05 - 0.21 \log DM$; $r^2 = 0.86$, $P < 0.001$.

$WC > WC_{\min}$, i.e. the time until air-dry). Fig. 2 shows the changes in the water contents of 11 cushions of different sizes in a climate chamber. The smallest sample (0.0026 g DM) lost all its water in *c.* 1 h, whereas the largest cushion (1.3 g DM) needed more than a day under the given conditions. Irrespective of temperature, the rates of water loss were initially high and constant for each cushion, but later (when WC was lower than *c.* 20% of the maximum water storage capacity) decreased continuously until all water was lost, in other words when WC_{\min} was reached. Very similar results were obtained in four additional runs at other temperatures and Δw values. Although the patterns of water loss were qualitatively similar (results not shown), the time necessary to reach WC_{\min} increased considerably to up to 40 h in

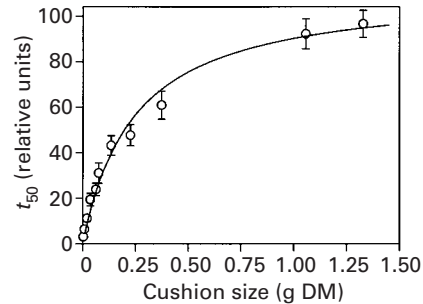


Fig. 4. Duration necessary to lose 50% of the water content of a saturated cushion (t_{50} , in relative units) as a function of plant size (dry weight, DM). Results are means \pm SD, $n = 5$. The regression line is $t_{50} = 462 DM (1 + 4.1 DM)^{-1}$; $r^2 = 0.98$.

large cushions. For all specimens of 0.02 g DM or more, an initial period of 3 h (27°C) to 6 h (4°C) was used to estimate the maximum rate of evaporative water loss per unit surface area (S , estimated from DM by using the equation for surface areas of wet cushions; see Table 1). The two smallest cushions were not included, because (1) the equation for S is valid only for larger cushions (Table 1) and (2) the fast loss of water would not have allowed enough weighings for a reliable estimate of the initial rates of water loss.

Maximum rates of evaporative water loss (E_{\max}) increased significantly with Δw (Spearman rank correlation, $P = 0.04$; $n = 5$). In smaller samples (0.02 g DM), E_{\max} averaged 220 mg of water m⁻² s⁻¹ in the five treatments (range 168–260 mg of water m⁻² s⁻¹). A comparison of E_{\max} and moss size (DM) revealed that the much faster depletion of cushion water in smaller than in larger specimens, as demonstrated in Fig. 2, was not only a consequence of a smaller amount of water stored per unit surface

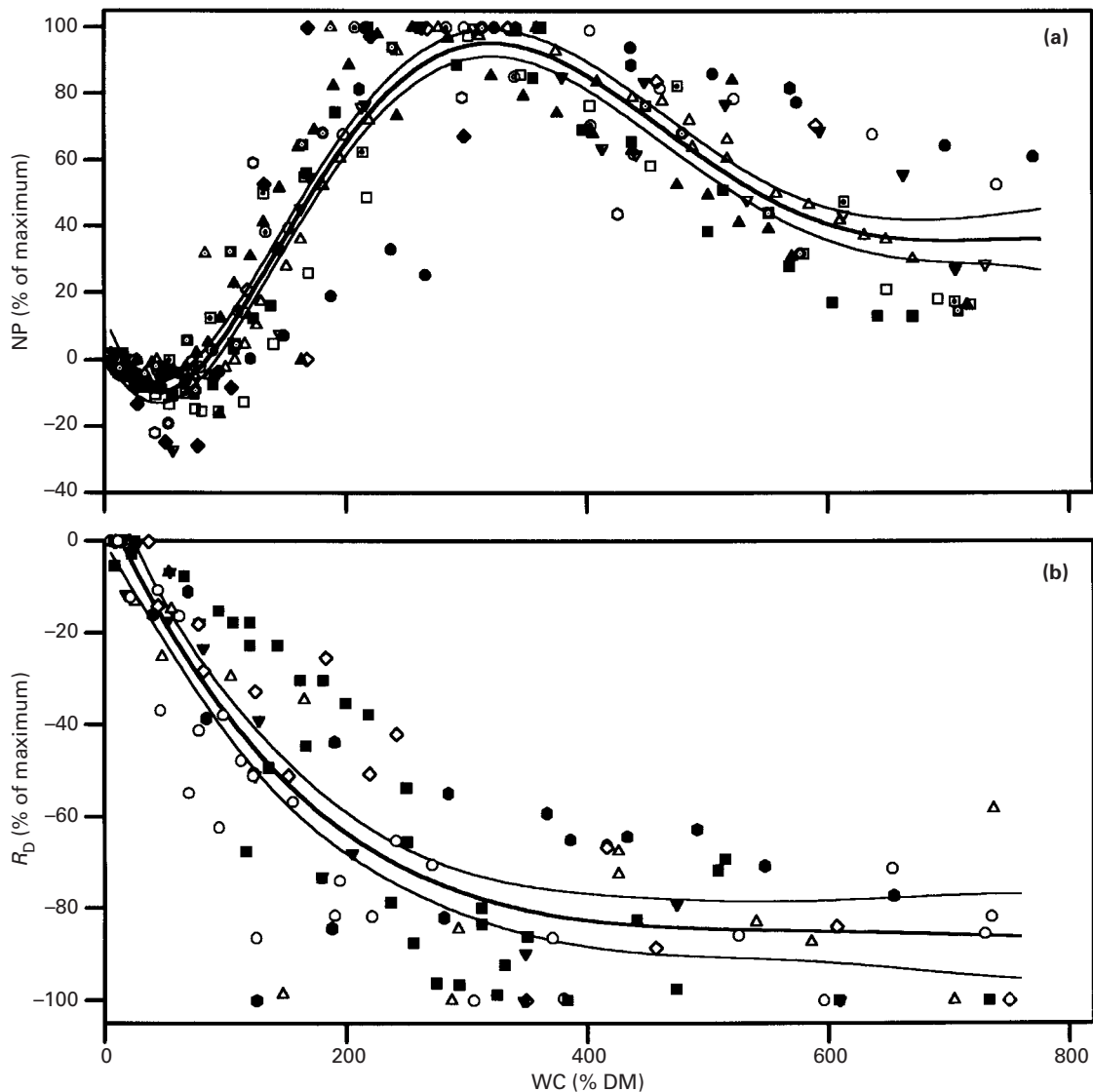


Fig. 5. Effect of changes in cushion water content (WC) on net photosynthesis (NP) and dark respiration (R_D) (as percentages of maximum). Different symbols represent different cushions. Measurements were performed at saturating PFD ($900 \mu\text{mol m}^{-2} \text{s}^{-1}$) and 21°C ($n = 27$ cushions) (a) or in darkness at 16°C ($n = 14$ cushions) (b); the size range was 0.03–1.1 g DM. The solid lines are sixth-degree (a) and fourth-degree (b) polynomial regressions ($r^2 = 0.85$ and 0.76 , respectively).

area, but was also due to much higher area-related evaporation rates (Fig. 3). For example, the approx. six-fold longer activity period of a moss of 1.3 g DM than for a moss of 0.02 g DM (2000 min compared with 340 min; Fig. 2) can be explained as the result of an S/V 3.3-fold higher (Fig. 1) and an area-related evaporation rate 2.7-fold higher (E_{max} ; Fig. 3) in the smaller moss cushion. In turn, these size-related differences in E_{max} were probably simply the consequence of an increased boundary-layer thickness in larger cushions. Proctor (1980) showed that smooth moss cushions at a low wind speed behave very similarly to a smooth plane surface in laminar flow. Thus, we can treat moss cushions as a flat, smooth surface, where the boundary-layer thickness (δ) increases with the square root of the diameter (Nobel, 1991). Calculating the expected δ from the diameters of the two cushions in question (10 and 44

mm, respectively) yielded a twofold difference in E_{max} , which compares favourably with the observed differences in evaporation rates.

We further analysed our data by determining the time required to lose 50% of the initial water content (t_{50}) for all samples during each of the five replicate runs. As shown in Fig. 4, this indicator of the length of metabolic activity was correlated with DM in a curvilinear fashion: t_{50} increased rapidly from the smallest sample to intermediate-sized samples, but only very moderately from intermediate to large samples. Large cushions at low Δw (i.e. 0.4 kPa) needed a t_{50} of up to 14 h (Fig. 4).

Net CO_2 gain and size

The qualitative response of NP to changes in WC did not differ between cushions of different sizes.

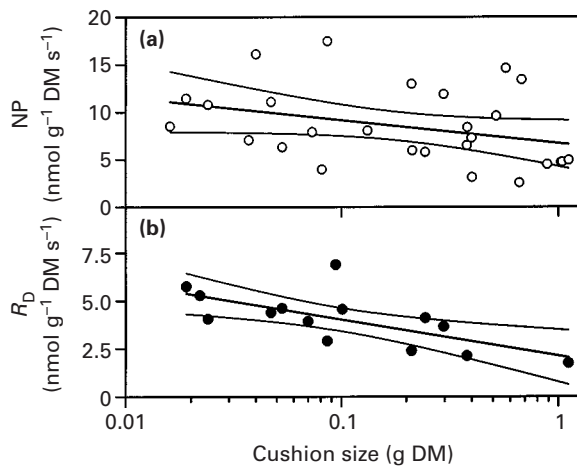


Fig. 6. Relationships of net photosynthesis (NP) (a) and dark respiration (R_D) (b) to cushion size (dry weight, DM). Solid lines are linear regressions; dotted lines are 95% confidence intervals. Regression equations are: $NP = 6.73 - 2.43 \log DM$; $r^2 = 0.12$, $P = 0.048$, $n = 27$; $R_D = 2.13 - 1.88 \log DM$, $r^2 = 0.47$, $P = 0.007$, $n = 14$.

There was no size-related difference in the WC at maximal NP ($r = -0.08$; $P = 0.69$; $299 \pm 72\%$ DM, mean \pm SD; $n = 27$), nor in the WC in which NP reached 0 $\text{nmol m}^{-2} \text{s}^{-1}$ ($98 \pm 29\%$ DM, $r = 0.17$; $P = 0.38$; mean \pm SD; $n = 27$), nor in the relative depression of NP at supraoptimal WC. For example, an increase in WC of 250% DM beyond

the WC at maximal NP resulted in an average decrease in NP of *c.* 50% ($56 \pm 17\%$, $r = -0.07$; $P = 0.74$; mean \pm SD; $n = 27$). Thus, we were able to describe the relationship of NP and WC with a single polynomial regression (Fig. 5a). However, there were size-related differences in the absolute rates. In spite of a considerable scatter, we found a significant negative correlation of NP_{\max} and cushion size ($r = -0.38$, $P = 0.048$, $n = 27$, range 2.6–17.4 $\text{nmol CO}_2 \text{g}^{-1} \text{DM s}^{-1}$; Fig. 6a).

In an analogous manner to our observations of the CO_2 gas exchange at saturating PFD, there was no size-related variation in the qualitative response of dark respiration to changes in WC (Fig. 5b), but the absolute rates of dark respiration decreased significantly with the size of a cushion (R_D , $r = -0.74$, $P = 0.003$, $n = 14$; Fig. 6b).

The above gas exchange data and the information on the kinetics of evaporative water loss (Fig. 2) allowed us to estimate the effects of size-related differences (1) on activity periods and (2) on the rates of NP and R_D and the C budget of a moss cushion. Interested in a somewhat realistic model, we chose a daytime temperature of 21°C (Δw 0.8 kPa) and night-time temperatures of 16°C (Δw 0.5 kPa) to simulate conditions of early summer in central Europe. Clearly, for the sake of the overall argument, we are considerably simplifying the complexity of conditions *in situ*. For example, we use the kinetics of

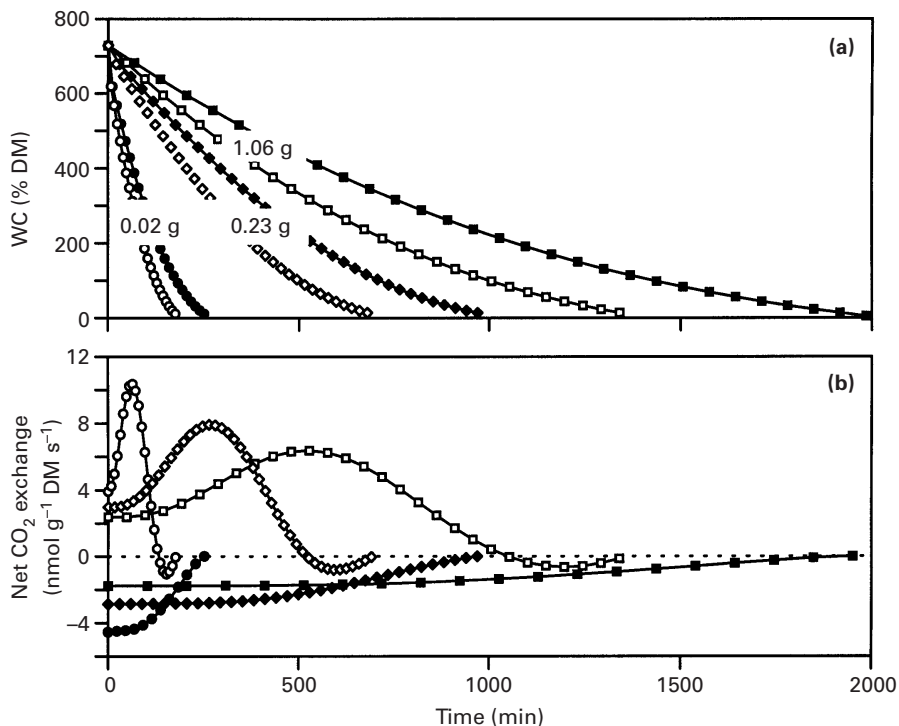


Fig. 7. Measured time courses of cushion water content (WC) during a drying cycle and estimated rates of net CO_2 exchange at saturating PFD (open symbols, $900 \mu\text{mol m}^{-2} \text{s}^{-1}$, 21°C, Δw 0.8 kPa) or in darkness (filled symbols, 16°C, Δw 0.5 kPa). Calculations were performed for three different cushion sizes, namely 0.02 g dry weight (DM) (circles), 0.23 g DM (diamonds) and 1.06 g DM (squares). For each value of WC, the net rate of CO_2 exchange was calculated from polynomial regressions similar to those shown in Fig. 5 and the correlation of NP_{\max} and dark respiration (R_D) with cushion size (Fig. 6).

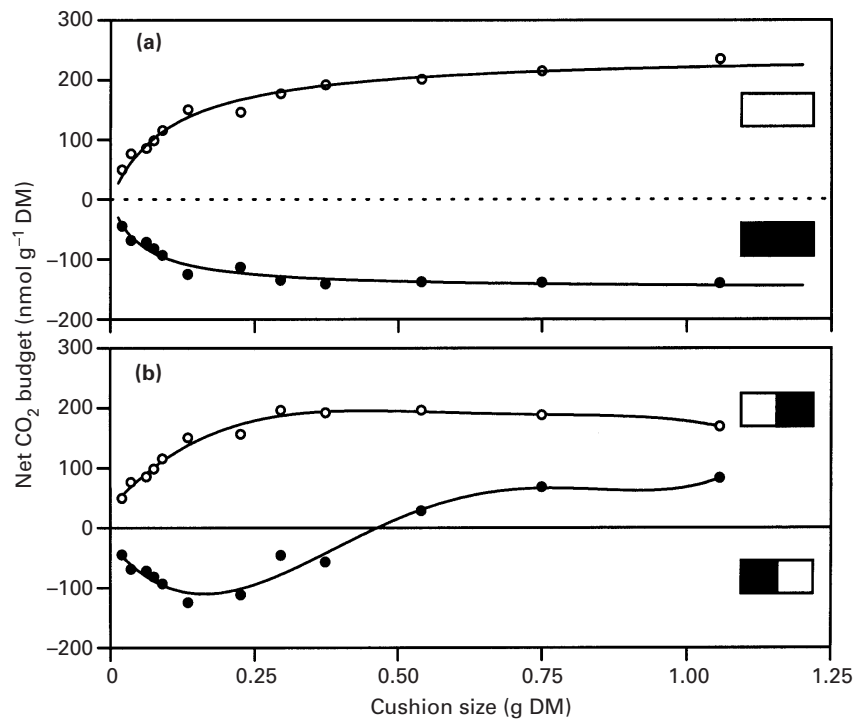


Fig. 8. Relationship between the integrated CO₂ budgets and an entire drying cycle and cushion size (dry weight, DM) (conditions as in Fig. 7). (a) Continuous light and continuous dark; (b) 12-h light–12-h dark and 12-h dark–12-h light rhythms. Lines are best-fit nonlinear regressions (in (a), $y = ax(1 + bx)^{-1}$; in (b), fourth-order polynomial regressions).

water loss obtained in low light (compare with Fig. 2) for conditions of saturating PFD or assume a rather small difference in Δw between day and night. However, because we are primarily interested in relative changes of C gain or loss with cushion size, the approach seems appropriate.

The results of three of these simulations are shown in Fig. 7 for cushions of 0.02, 0.23 and 1.1 g DM, respectively. A full recharge ($WC = 730\%$ DM) allowed much longer activity periods in the dark than in the light. As expected, larger cushions were active for considerably longer periods but reached lower rates of net CO₂ exchange both in the dark and when illuminated. In a similar fashion, we computed the C gain or loss, respectively, for a total of 11 cushions in a size range from 0.002 to 1.1 g DM. The lengths of the activity periods (results not shown), and both C gain and C loss increased in a curvilinear manner with plant size (Fig. 8a). Notably, relative size-related changes were most pronounced at smaller sizes (<0.2 g DM), whereas the estimated CO₂ budgets were almost independent of size in larger cushions. This simple relationship was lost when we included alternations of light and dark phases as an additional element in the model calculations. We assumed two models: recharging of the cushions with water at the beginning of a 12-h light period or at the beginning of a 12-h dark period (Fig. 8b). In the first case, all cushions of >0.25 g DM were active for >12 h. Hence, the highest CO₂ gains of a complete drying cycle could be observed in

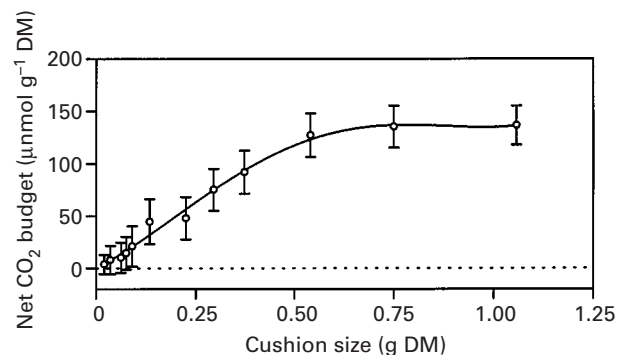


Fig. 9. Average carbon budgets of an entire drying cycle as a function of cushion size (dry weight, DM) (conditions as in Fig. 7). Shown are means \pm SE for 24 determinations, starting at hourly intervals through the 24 h. The line represents a best-fit fourth-order polynomial regression. Note the assumption that rainfall probability is independent of the time of day.

cushions of intermediate size, whereas the C budgets of larger sizes were slightly decreased owing to respiratory CO₂ losses. Wetting at the beginning of the dark phase resulted in a more complicated pattern. Small cushions (<0.1 g DM) dried out during the dark phase, intermediate sizes (0.1–0.6 g DM) were active both in the dark and the light, and the largest cushions (>0.6 g DM) were active for >24 h. Consequently, a size-related decrease in the net CO₂ budgets was followed first by an increase and then by a relatively stable level of C budgets in the largest cushions. Finally, Fig. 9 shows the results

of 24 similar computations of cushion C balances, but starting fully hydrated at hourly intervals round the 24 h. Again, there was a strong size effect in smaller cushions, whereas those of >0.5 g DM did not differ in their net CO_2 budgets.

DISCUSSION

Size differences in moss cushions of a given species, *Grimmia pulvinata*, cause pronounced differences in their water relations (Figs 1, 2, 3). In spite of a general understanding of the importance of 'life form' (Gimingham & Robertson, 1950; Gimingham & Birse, 1957; Bates, 1998) and the repeated calls for attention to the importance of size in poikilohydric organisms (Larson, 1984; Gauslaa & Solhaug, 1998) researchers have paid relatively little attention to possible adaptive changes in form and function on a smaller, intraspecific scale. The S/V of a smaller body is inevitably larger unless changes of form occur (Niklas, 1994). In our study species, smaller cushions are almost perfect hemispheres, whereas larger ones are increasingly flattened. This change in plant shape must result from a particular pattern of growth and development of the entire cushion. Whether this pattern is indeed the outcome of past selective processes or just a structural necessity remains a subject of speculation (Gould & Lewontin, 1979), but the consequences of this allometric change are unequivocal; the relative decrease in surface area in smaller individuals allows somewhat longer periods of metabolic activity than would otherwise be possible. Assuming adaptiveness, the question arises why larger individuals should not have evolved a different shape to maximize their activity periods by approaching a hemispherical form. We suggest that the advantage of prolonged activity would be counterbalanced by an increase in nonproductive biomass in the lower parts of the cushions; light penetrates only a few millimetres into these dense cushions (G. Zotz, unpublished). An already increasing proportion of such 'brown tissue', which is still alive (Longton, 1972), is probably responsible for the decrease in mass-based rates of net CO_2 exchange with size in *G. pulvinata* (Fig. 6).

However, most of the change in S/V is simply a consequence of allometry (Niklas, 1994). Larger individuals have an advantage over smaller conspecifics in two respects; they store more water for each unit cushion surface (Table 1), and they have a higher boundary-layer resistance (Gates, 1980). The resulting increase in the time of metabolic activity in poikilohydric organisms is often equated to C gain and growth (e.g. Proctor, 1972; Pérez, 1991, 1997; Gauslaa & Solhaug, 1998). Although activity periods and C budgets were indeed closely correlated under constant conditions, our model calculations (Figs 7, 8) indicate that such a view is too simplistic. Clearly, the effect of size on the C gain of a cushion in the

course of a drying cycle strongly depends on the time of wetting, and longer activity periods might equally well correspond to more C gain or more C loss (Figs 7, 8). Introducing light-dark cycles into our considerations might lead to rather complex patterns of the relation between cushion size and integrated C budget (Fig. 8b). Recharging a cushion with water at the beginning of the light period of a 12-h light-12-h dark cycle leads to strong increases in C gain at smaller sizes, whereas in larger cushions higher gains in the light period are counterbalanced by increasing losses during extended activity in the dark, which leads to a rather constant value of integrated C gain in larger cushions. Rewetting at the beginning of the night results in C budgets ranging from *c.* -100 to *c.* $+100 \mu\text{mol s}^{-1} \text{g}^{-1} \text{DM}$ (Fig. 8b). Clearly, our model calculations of the wetting of a cushion could yield almost any kind of quantitative result when the entire size range is included.

At present, these calculations admittedly cover just a small portion of possible environmental conditions. However, at our study site in the Würzburg Botanical Garden, even larger cushions (>0.5 g DM) were affected considerably and throughout the year by low water contents: photosynthetic activity ceased on average for *c.* 20% (autumn) to 50% (summer) of total daytime during a 1-yr field study of the CO_2 gas exchange of *G. pulvinata* (S. Rottenberger & G. Zotz, unpublished). Given other climatic conditions, for example the mild and moist winters of southern England (Proctor & Smith, 1995), cushions of *G. pulvinata* and other species might stay wet and active for extended periods; however, this is not so in central Europe, where winters are much harsher and cushions are frequently frozen and inactive (S. Rottenberger & G. Zotz, unpublished). Conversely, in sunny weather in summer, activity periods might be much shorter than those in our model calculations. For example, during preliminary measurements of the *in situ* gas exchange and water relations of *G. pulvinata* in late June and July, small, fully exposed cushions (0.1 g DM) dried out in <4 h after hydration at dawn, whereas larger specimens (1.1 g DM) were active for almost 9 h, with intermediate values for intermediately sized cushions (G. Zotz, unpublished). This suggests that, under the climatic conditions of central Europe, size-related differences in metabolic activity can be observed throughout the year in a consistent manner. Remarkably, however, this seems to be true only for cushions smaller than 0.5 g DM. Among the largest individuals, differences in the net CO_2 budgets were negligible (Figs 8, 9).

The finding of size dependence in a number of water relations parameters such as cushion shape (Fig. 1) or water loss kinetics (e.g. t_{50} , positively correlated with size; Fig. 4), and gas-exchange parameters such as NP_{max} (negatively correlated with size; Fig. 6) is important in the context of

bryophyte physiology in general. However, our results are probably particularly relevant when data are used to assess the physiological basis of ecological observations such as species distribution. For example, a study with the congeneric *G. laevigata* (Alpert, 1988) suggests that observed micro-distributions are determined by establishment rather than by adult survival. In this case, studies with large individuals (or with individuals of unspecified size) would be of little relevance, assuming that changes in physiological characteristic as are found in *G. pulvinata* are indeed a general phenomenon, at least in cushion mosses. The observation of Alpert (1988) is not exceptional: in studies with several species of lichen, physiological differences in thallus size were reported repeatedly (Hestmark *et al.*, 1997; Gauslaa & Solhaug, 1998; Fos *et al.*, 1999). All these observations add to the increasing evidence that size has to be treated as an essential parameter in physiological and ecological studies, not only in poikilohydric organisms but in plants in general (Donovan & Ehleringer, 1992; Gauslaa & Solhaug, 1998; Zotz, 2000).

In conclusion, studies dealing with water relations or net CO₂ exchange of bryophytes should adequately address the effect of size on physiological parameters, or at least be specific about the sizes of the specimens used. Merely the choice of specimen size of, for example, two species of cushion moss might determine the outcome of a comparative study. Moreover, when physiological parameters such as NP_{max} or R_D are size-dependent, studies of the C gain *in situ* with large individuals might be of little relevance to the study of distributional limits if establishment limits distribution. It is clearly worse, however, when size is not specified at all because later unequivocal interpretation of such data is virtually impossible.

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