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Research Article

An Experimental Approach to Examining the Effect of Water Depth and *Lemna minor* L. on Algal Growth

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Abstract: In order to test how both water depth and *Lemna minor* L. affect the growth rate of algae, two different experiments were designed. In the first experiment, *Scenedesmus quadricauda* (Turp.) Breb. was cultured in sterilized Steinberg solution with reduced N and P levels in plastic containers at depths of 5, 10, 15 and 20cm. At the end of the experiment, differences in algal growth with depth were found to be significant between a) 5 cm with 10 cm, 15 cm and 20 cm, and b) 10 cm with 15 and 20 cm depth treatments ($p<0.001$).

In the second experiment, *L. minor* was set up without (group I) and with (group II) *Scenedesmus quadricauda* and at a constant water depth. *L. minor* was added to the containers to create treatments with starting covers of 0, 10, 30, 60 and 100% to produce different initial light levels for the algae. At the end of the experiment, algal growth was found to be reduced by the presence of *L. minor*, the reduction being positively correlated with increasing cover percentage of *L. minor* ($p=0.017$) in group I. Although mean algal chlorophyll increased very slightly with increasing cover percentage, the effect of *L. minor* on algal growth was not significant in group II.

Key Words: water depth, algae, floating plant, *Lemna, Scenedesmus*

Su Derinliği ve *Lemna minor* L.'un alg Büyümesine Etkilerinin Deneysel Olarak İncelenmesi

Özet: Su derinliğinin ve yüzen su bitkisi *Lemna minor* L.'un alglerin büyüme oranlarına etkilerini test edebilmek için iki farklı deney düzeneği hazırlanmıştır. İlk deneyde *Scenedesmus quadricauda* (Turp.) Breb. sterile edilmiş ve düşük N, P değerleri içeren Steinberg solusyonunda 5, 10, 15 ve 20 cm derinlikte plastik kaplara kültür edilmiştir. Deney sonunda a) 5 cm ile 10, 15 ve 20 cm b) 10 cm ile 15 ve 20 cm derinlikler arasında istatistiksel olarak anlamlı farklılıklar bulunmuştur ($p<0.001$).

İkinci deneyde yüzen su bitkisi *Lemna minor*, *Scenedesmus quadricauda* yokluğunda (grup I) ve varlığında (group II) sabit su derinliğinde büyütülmüştür. *L. minor* alglere farklı miktarlarda ışık sağlamak amacıyla deney kaplarına başlangıç için yüzde 0, 10, 30, 60 ve 100 lük örtme oranlarıyla eklenmiştir. Deney sonunda I. Gruptaki alg büyümesi *L. minor*'un örtme yüzdesinin artışına paralel olarak azalmıştır ($p=0.017$). Artan *L. minor* örtüsüne bağlı olarak ortalama algal klorofilin belirgin bir şekilde yükselmesine rağmen II. Gruptaki alglerin büyüme oranı istatistiksel olarak anlamlı bulunamamıştır.

Anahtar Sözcükler: su derinliği, alg, yüzen bitki, *Lemna, Scenedesmus*

Introduction

The relative importances of nutrient and light limitation on algal growth vary with depth. Algae are more likely to be nutrient limited when they receive the high light intensity available at the top of the water column and have potentially higher growth rates, and are relatively more light limited when in the low light regions towards the bottom of a column (Huisman & Weissing, 1995).

The aim of the first experiment was to investigate the effect of water depth on the algal growth in a system with floating *L. minor* L. cover. Water depth will of course vary in natural ecosystems where *L. minor* and algae are interacting and it is important to know how the interaction will operate at different depths.

Macrophytes provide a large surface area for colonization by algae and bacteria and a significant portion of the vegetative biomass in the littoral zone is contained in the epiphytic cover (Bronmark, 1989). Epiphyton consists of a structurally complex assemblage of living algae and bacteria, senescent and dead cells, detritus and particulate calcium carbonate crystals

($CaCO_3$), embedded in a matrix of polysaccharide and protein complexes originating from both the macrophytes and the epiphyton (Allonson, 1973). Thus a symbiotic relationship has been suggested to exist between aquatic macrophytes and their bacterial and algal epiphytes (Wetzel, 1983). The epiphytic microflora is suggested to utilize nutrients and dissolved organic matter (DOM) excreted by the macrophytes (Bronmark, 1985), while the algal layer may be advantageous to the macrophyte by providing it with protection from herbivory, by diverting grazers away from the macrophyte tissue (Hutchinson, 1975).

Epiphytic species may also be nutritionally supported by floating macrophytes in the same way that they are supported by submersed macrophytes, i.e., by released dissolved organic materials. It is claimed that species of *Lemnaceae* can provide sugars and other organic substances for other organisms (Wetzel and Manny, 1972), and that this may partly explain why many small organisms (e.g., cyanobacteria and eubacteria) are physically associated with species of *Lemnaceae*.

On the other hand, the autotrophic green algae associated with *L. minor* have no known nutritional relationships with the macrophyte. For them, the plant may simply provide a surface and dense covers may be deleterious in that they restrict light for photosynthesis of the algae.

However, algae are competitive with *Lemnaceae* in nutrient rich waters. Very often a thick algal cover is physically raised by the development of large gas bubbles, thus breaking the contact of the duckweed plants with the water, resulting in the drying of fronds (Landolt, 1986). On the other hand, heavy dense covers of *Lemnaceae* reduce light penetration and gas exchange and thus can inhibit algal growth.

It is possible therefore that where algae are associated with floating plants in significant numbers there may be stimulation of the floating macrophytes, which in turn may lead to suppression of the growth of submersed macrophytes. On the other hand, competitive growth of algae may restrict the dominance of floating macrophytes, which in turn will allow more growth of the submersed vegetation, if the algae do not themselves create conditions unfavourable to the submersed component. It will often be a fine distinction which determines which way the ecosystem goes. The aim of the second experiment was to explore the interrelationships further by varying both the densities of the floating plants per unit water surface and the algal abundance in the water on which the macrophyte is growing.

Materials and Methods

In the first experiment, populations of *L. minor* and alga were grown in open-topped plastic bottles of different depth. A total of 20 plastic, 2 litre, 10cm diameter bottles were cut to four different depths, using scissors. This provided each depth treatment (5cm, 10cm, 15cm, 20 cm deep), each with five replicates.

Scenedesmus quadricauda (Turp.) Breb., which is often present in field situations with *L. minor*, was used as the added test alga in case the natural algal flora associated with *L. minor* was insufficient to create effects within a reasonable experimental time span. *Scenedesmus quadricauda* is a common, widely distributed tychoplankter of shallow, eutrophic waters. It was obtained from a stock culture from the Cambridge Culture Collection of Algae and Protozoa (CCAP), (isolator Jaworski 1984, origin Freshwater; Priest Pot, Cumbria, England and culture Medium JM; liquid and agar; B). Full Steinberg Solution (Steinberg, 1941) has very high N and P levels, which would stimulate algal growth unnaturally. Stock culture of alga was therefore added at approximately 1ml to 1 litre, to sterilized Steinberg Solution with reduced N and P levels, approximately 40 mg l^{-1} N and 5.7 mg l^{-1} P. Each plastic container was filled with this reduced N and P Steinberg Solution: algal suspension mix. Since *L. minor* was expected to grow during the experiment, the starting number was 30 individual plants per container (a 20% cover) with *L. minor* plants, taken from the stock culture.

The experiment was carried out in a 15°C constant temperature room over 21 days. The light irradiance was approximately 55 µmol PAR $m^{-2}s^{-1}$, measured with a Macam Quantum Radiometer/Photometer Q101 (Macam Photometrics Ltd., Livingston, Scotland) underwater probe, and a 12:12 hours light:dark cycle was used.

As a precaution, pH measurements were performed at the beginning, at the end, and twice between the beginning and the end of the experiment using a Camlab pH Boy-P2 pH probe (Camlab Ltd., Nuffield Road, Cambridge), so that any extreme or unexpected changes

in the cultures would be detected. *L. minor* plants were counted five times during the experiment. Algal growth in each container was determined at the end of the experiment from the algal chlorophyll content of a sample of the suspension after scraping any attached algae from the walls and then the total algal content was calculated from the volume of container x the algal chlorophyll per unit volume. Chlorophyll analysis was performed according to Arnon (1949).

In the second experiment, mixed cultures of the alga *Scenedesmus quadricauda* and *L. minor* were set up. The *Scenedesmus quadricauda* was used in case the *L. minor* was carrying little or no algal population of its own. Populations of *L. minor* were cultured on Steinberg Solution with reduced N and P as in the first experiment. A total of 50, 4 cm high and 6.5 cm diameter glass containers were used. The outsides of the containers were covered by aluminium foil before being filled with sterilized Steinberg Solution. The experiment involved two groups: group I with 25 containers with only Steinberg Solution and group II with 25 containers with Steinberg Solution + algal inoculum. *Scenedesmus quadricauda* was added to the culture solution at a rate of 1ml to 1 litre. The alga used was from pure stock culture as in the first experiment.

L. minor plants were added to containers to create treatments with starting covers of 0, 10, 30, 60 and 100% to produce different initial light levels for the algae. Each percentage cover treatment had five replicates. The required number of *L. minor* plants was counted into each container.

The experiment was conducted in a 15°C constant temperature room for 21 days. The light irradiance was approximately 97.5 µmol PAR m^{-1} s^{-2}, measured by using the same equipment as in the first experiment and a 12:12 hours light:dark cycle was used.

Moreover, pH was measured three times during the experiment as a check on any unexpected or extreme change in the system. *L. minor* was counted and total algal chlorophyll was determined for each container, using the method of Arnon (1949). Samples of the culture medium from each container were also examined by light microscopy to determine which algal species had grown.

In both experiments, no circulation system was used in the containers, to avoid disturbing *L. minor* on the surface.

Results and Discussion

In the first experiment, the pH of culture solution increased during the experiment, presumably due to algal growth (Fig. 1a). The increase in pH was rapid until day 10 in all containers and then the increase continued in 5 and 10cm depth containers, but pH was then almost stable in 15 and 20cm depth containers. In shallow containers (5 and 10cm depth) more algal growth per unit volume occurred than in the deep containers and this is probably why pH increases were greater in these containers. pH changes, however, were not excessive, such as to create unfavourable conditions during the experiment.

In the second experiment, in both groups, the pH in the medium increased during the experiment, with 10, 30, 60 and 100 cover percentages, but was almost constant with zero cover percentage (Figs. 1b, 1c).

L. minor grew well in each depth of container in the first experiment (Fig. 2a). It was green and healthy and had fronds of normal size (4mm diameter). In each container it had very long roots. *L. minor* also grew well in all containers, though the relative increase was lower at higher starting cover percentages in the second experiment group I, with no algae added to the system (Fig. 2b), and group II, with *Scenedesmus* Meyen added to cultures (Fig. 2c). It was green and healthy and had large fronds (approx. 5mm diameter). It had long roots (approx. 40mm), typical of roots produced by *L. minor* in media with some nitrogen limitation and/or high light intensity (Table 1).

Algal growth (Fig. 3a) was calculated as total algal content for each depth of container. It was found to be highest for 5cm depth containers, with the difference from other treatments statistically significant in the first experiment. After 10 cm depth, total algal content sharply decreased, and 15cm and 20cm depth containers had similar, not statistically different, amounts of alga. Differences in algal growth with depth were significant between i) 5cm with 10cm, 15cm and 20cm and ii) between 10cm with 15cm and 20cm depth treatments ($p<0.001$).

The situation regarding the distribution of algae with depth was different for different species. Microscopic investigation of the cultures showed that chaetophoralean algae and *Chlamydomonas* Ehrenberg sp. were dominant in containers 5cm and 10cm deep while Scenedesmus sp.

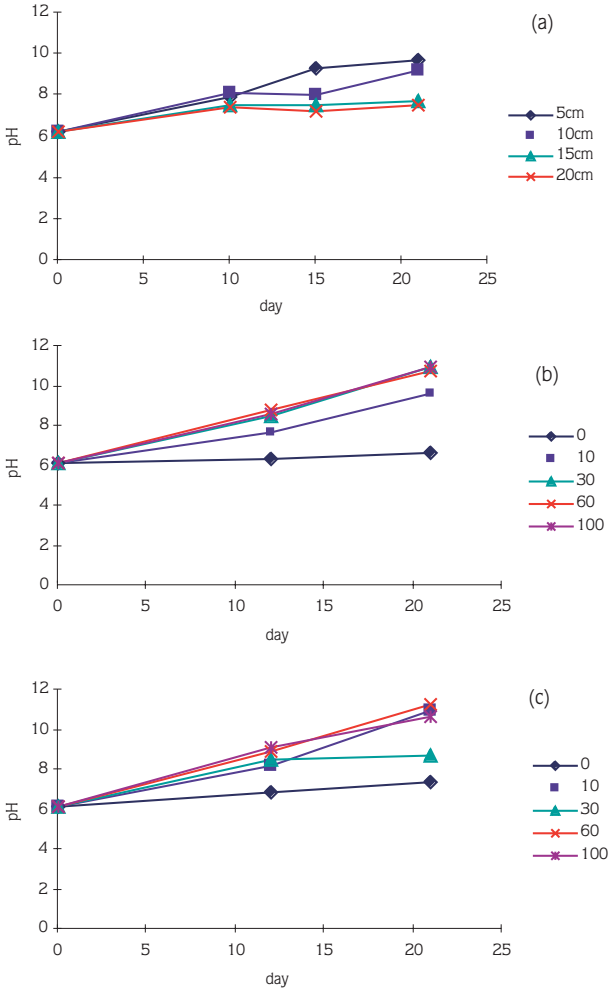

Figure 1. pH against time (a) under different depth growth conditions, (b) group I and (c) group II treatments over 21 days.

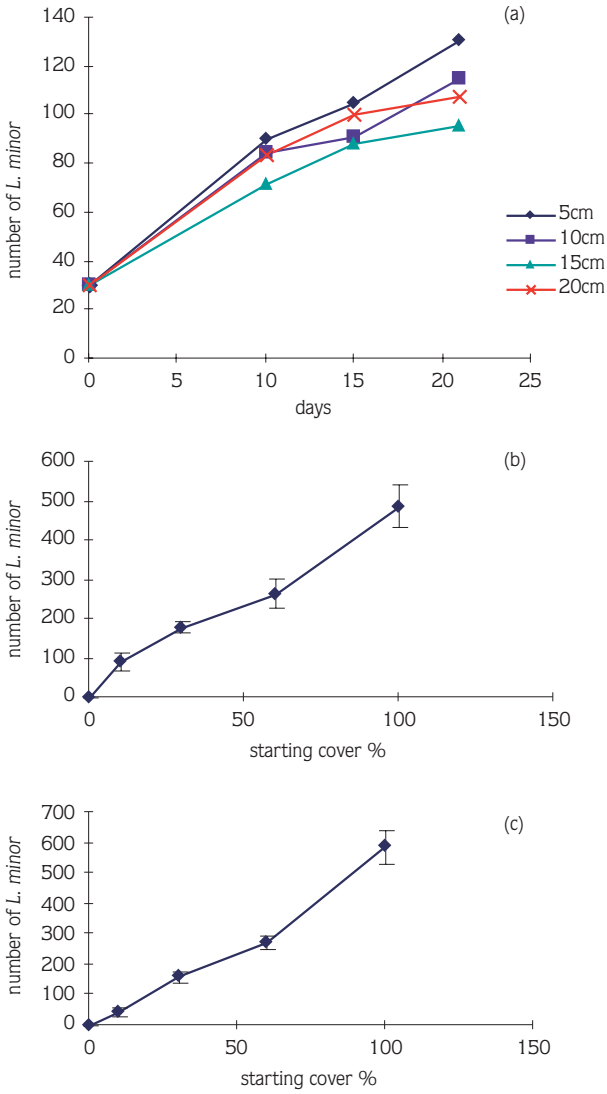

Figure 2. Number of L. minor plants against (a) the time under different depth growth conditions and the starting cover percentage (b) for group I and (c) for group II treatments over 21 days.

was frequent. In contrast, *Scenedesmus* sp. was dominant in 15cm and 20cm containers and the other two types, chaetophoraleans and *Chlamydomonas* sp., were frequent. Chaetophoralean algae and *Chlamydomonas* sp. must have arisen from the *L. minor* fronds.

Nutritional factors may influence algal growth and distribution. According to Tilman's resource competition theory (Tilman, 1977), at or near a competitive equilibrium, species dominance and relative abundance are determined by the ratio of the limiting nutrients, i.e., Si : P, Si : N. Two species can coexist at a steady state when each is limited by a different resource, e.g., species

starting cover %	G I cover % at 21 days	G II cover % at 21 days
0	0 (0)	0 (0)
10	115 (13.90)	57 (8.6)
30	216 (14.90)	200 (11.7)
60	330 (24.17)	388 (13.3)
100	609 (34.93)	742 (32.0)

Table 1. Increase in *L. minor* with (G II) and without (G I) *Scenedesmus quadricauda* addition, over 21 days. Values shown are means (n=5) with standard errors in parentheses.

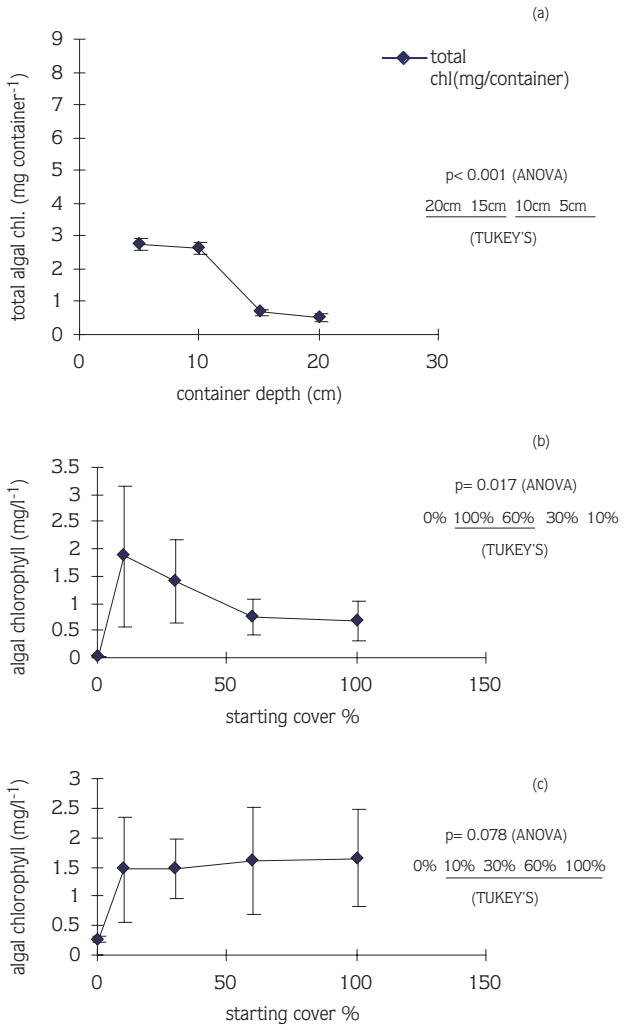

Figure 3. Algal chlorophyll against (a) the different depth containers and the starting cover percentage (b) for group I and (c) for group II treatments over 21 days.

A is limited by nutrient X while species B is limited by nutrient Y (Steward and Levin; 1973, Levins, 1973).

Steinberg Solution, which contains relatively high N and P levels, was used in this experiment. It seems unlikely that algal growth could be limited by nutrient availability. On the other hand, nutrient levels of the solutions were not measured at the end of the experiment. It is possible that nutrient levels of the solutions could be reduced by the *L. minor* which grew on the surface of the solutions. Therefore nutrient limitation for algae could take place in the containers. Light is also an important factor for algal growth. According to Huisman and Weissing (1995), in a very shallow mixed water layer, light is readily available but the total amount of nutrients is very low. As a consequence, nutrients are easily depleted and total biomass remains low. An increase in the mixing depth first raises the total amount of biomass because more nutrient becomes available for growth. However, great mixing depths of water (around 100 m) usually lead to a low average light exposure of algal cells circulated through the vertical light gradient (Sommer, 1988), which reduces total biomass (Huisman and Weissing, 1995). In this experiment, however, the surface of the water was covered by *L. minor* and therefore light penetration would also be affected by this macrophyte layer. Thus algal growth could potentially be restricted by reduced light. With increasing depth of container, the mean amount of light radiation received per alga will decrease. Hence it is not surprising that chlorophyll per unit volume decreases with increasing container depth. Chaetophoralean algae release phototactic motile spores which probably attached selectively to the upper, better illuminated parts of the culture system. *Chlamydomonas* sp. is similarly motile and phototactic and would tend to position itself in the upper parts. *Scenedesmus* sp., by contrast, is immotile and lacks attachment mechanisms, so it would be most likely to accumulate on the bottoms of the containers.

Microscopic investigation showed that mainly epiphytic species *Oscillatoria* Vaucher sp., *Chlamydomonas* sp. (green), a chaetophoralean (green), and *Trachelomonas* Ehrenberg sp. (euglenophyte) developed in containers when only *L. minor* was present at the start of the experiment.

starting cover % of *L. minor*	G I algal chlorophyll (mg l^{-1}) at 21 days	G II algal chlorophyll (mg l^{-1}) at 21 days
0	0.03 (0.004)	0.28 (0.025)
10	1.88 (0.663)	1.47 (0.460)
30	1.41 (o.387)	1.49 (0.260)
60	0.77 (0.165)	1.62 (0.470)
100	0.69 (0.181)	1.65 (0.420)

Table 2. Change in algal population density, expressed as algal chlorophyll, with (G II) and without (G I) added *Scenedesmus* over 21 days. Values shown are means (n=5) with standard errors in parentheses.

In the second experiment, as shown in Table 2 and Figure 3b, algal growth was found to be reduced by *L. minor*, the reduction being positively correlated with increasing percentage cover of *L. minor* for group I ($p=0.017$).

Algal growth was very low in the 0% cover container (algal chlorophyll 0.28 mg l^{-1} at the end of the experiment). On the other hand, the effect of *L. minor* on the algal growth in group II treatment (10 to 100% cover) was not significant ($p= 0.078$ ANOVA) (Fig. 3c), although mean algal chlorophyll increased very slightly with increasing percentage cover (Table 2). However, although only *Scenedesmus quadricauda* was added at the beginning, microscopic investigation showed that *Oscillatoria* sp., *Chlamydomonas*, chaetophoraleans and *Trachelomonas* were also abundant at the end of 21 days, having presumably been introduced into the system with *L. minor*.

L. minor grew to almost the same extent with and without *Scenedesmus* sp. being added to the culture solution. Algae did not, however, grow so well in group I treatments which reached high percentage cover (above 216%) of *L. minor* at the end of the experiment. Dense covers of *Lemnaceae* will reduce light penetration and gas exchange and thus can inhibit algal growth (Landolt, 1986).

In contrast in group II treatments, high percentage cover of *L. minor* did not suppress algal populations and the mean algal chlorophyll levels were even a little higher at the higher *L. minor* levels. The additional algal production in group II could be because of the larger starting inoculum (i.e., epiphytes + *Scenedesmus* sp). This starting inoculum could be increased by the high percentage cover of *L. minor*. Additionally, a mixed algal community, and even a single algal population, can sometimes be simultaneously limited by both light and nutrients over a narrow range of low irradiances (Healey, 1985). An alga growing in an environment with both low light and low N supply rates could potentially increase growth due to an increase in either irradiance or N supply rate. An increase in irradiance would result in a lower "requirement" for chlorophyll and other photosynthetic pigments, and the membranes to accommodate them, "freeing" nutrients such as N and P within the cells. This could then lead to a higher growth rate at the same internal N and P concentrations. Similarly, an increase in N and P under limiting nutrient flux conditions would allow more photosynthetic pigments and membrane to be produced, to allow more rapid growth at the same low light conditions.

Increases in pH in cultures over the 21 days were very similar between group I and II treatments and generally greater in 10, 30, 60 and 100 cover percentages than in zero cover percentage of *L. minor*. Rising pH could be due to photosynthesis of *L. minor* or of the algae, but the pH increase was almost the same in group I and II, even though twice as much algal growth occurred in group II. Morris and Barker (1977) observed an 18% supersaturation of oxygen in the water below the duckweed cover irradiated with 63 W m^{-2}. It is likely therefore that increasing pH was mostly due to photosynthesis of *L. minor*.

Conclusions

Total algal growth in the first experiment was affected by the depth of the containers and this could be due to epiphytic algae being limited by light and nutrients. *Scenedesmus* occurred mostly near the bottom of the containers, settling there as an immotile alga. *Chlamydomonas* and chaetophoralean algae, presumably derived from populations introduced as epiphytes on the *L. minor*, were motile, which enabled them to selectively colonize the upper, better-illuminated levels in the cultures.

The water surface of the containers was only 20% covered by *L. minor* at the start of the experiment, though this reached a maximum of 86% cover by the end of the experiment, and thus sufficient light would be available for algal growth throughout most of the experimental period. In contrast, in field conditions, with a more dense cover of *L. minor*, which is very possible, both epiphytic and planktonic-benthic algal growth could be affected by the greater light reduction.

However, *L. minor* did not seem to be affected by algal growth and the frond number increased by almost the same amount in each depth of container during the experiment. Once more it is clear that there are complex interactions between floating vegetation and algae within the ecosystem, both epiphytic and suspended components.

L. minor grew well and to almost the same extent with and without *Scenedesmus* added to the treatments

in the second experiment. It therefore seems that the alga does not affect the growth of *L. minor*, at least within the time range and population densities used in these experiments.

L. minor has an epiphytic algal flora of its own and this is capable of increasing in conditions of high light supply. Growth of these epiphytes was significantly reduced by higher cover percentage of *L. minor*, but *Scenedesmus* was not limited in this way and achieved dense populations beneath the highest percentage covers of *L. minor* recorded in these experiments. Greater *Scenedesmus* growth occurred as *L. minor* cover increased. This suggests that *L. minor* stimulated *Scenedesmus* growth, perhaps through producing shade conditions which suit this alga, although published literature (Wetzel and Many, 1972; Baker and Farr, 1987) suggests that production of organic algal growth stimulators is another possibility.

Within the population density limits of these experiments, it is evident that, although interactions between the macrophyte and algae are complex, the growth of the former is little affected by the latter, whereas the algae benefit considerably from the presence of the macrophyte. It seems that the benefit to the algae will be through the provision of surfaces for growth, but more subtle effects such as shading against excessive light may also be important.

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