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Review article

Energy and Nutrient Acquisition by Autotrophic Symbioses and Their Asymbiotic Ancestors

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Abstract

The hypothesis that macroscopic autotrophic symbioses involving microscopic autotrophs increase the rate of resource acquisition per unit substrate area relative to the microscopic autotrophic symbionts in isolation is examined for lichens and for symbioses involving invertebrates. Available data show that this rate is invariably increased in invertebrate symbioses, and is frequently increased in lichens. Since much of the photosynthate from symbiotic microalgae is transferred to the chemoorganotrophic partner, this does not inevitably lead to a higher rate of area-based algal production or of potential microalgal propagation in symbioses as compared to free-living microalgae.

The extent to which the pre-symbiotic properties of the chemoorganotrophic partner can account for the observed enhancement in the rate of resource acquisition relative to free-living micro-photobionts is considered. The conclusion is that a number of structural traits of subaerial hyphal aggregates of fungi, and of coelenterates and bivalves, provide an appropriate basis for a macroscopic phototroph involving a microscopic photobiont.

The resource-acquisition properties of the macroscopic symbioses are briefly compared with those of macrophytes produced by evolution of large acellular, or multicellular, phototrophs from microscopic unicells. The absence of homoiohydric lichens is commented on in the context of the apparently appropriate attributes of non-lichenised fungi.

Keywords: ammonium, bicarbonate, bivalves, carbon dioxide, chemosynthesis, Chlorophyta, Coelenterates, Cyanobacteria, Dinophyceae, iron, lichens, nitrate, phosphate, photosynthesis

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1. Introduction

The exploitation of solid substrate, whether submerged or subserial, by free-living unicellular or simple filamentous photolithotrophs appears to be incomplete in that available resources are not converted to biomass as effectively as is the case with macroscopic phototrophs exploiting similar habitats. This assertion is left unsupported by literature citations; one of the tasks of this paper is to provide evidence as to its validity by using the scattered literature comparing net photosynthetic (and nutrient incorporation) data for the benthic microalgae with those of benthic macrophytes. As well as the microphotobiont – fungus (lichen) or microphotobiont – invertebrate associations which are the main examples used in this paper, a comparison is also attempted between the benthic microalgae and the macrophytes produced by evolution of microalgae into, on the one hand, large acellular (coenocytic) algae (essentially all aquatic) and, on the other, multicellular algae and higher plants (aquatic and terrestrial): Raven (1986a).

The approach outlined above emphasizes the capacity of populations, or communities, to acquire resources, and of species or guilds to dominate communities. From the viewpoint of the evolutionary success of the microphotobiont it is also important to consider the potential for population increase of the microalga living in isolation in comparison with the potential when in symbiosis. The importance of this emerges from a consideration of the fate of photosynthate in the two situations; even with a higher area-based microalgal population density in symbiosis than for free-living microalgae, and a higher area-based rate of net photosynthesis of symbioses than of free-living microalgae, the diversion of photosynthate to the chemoorganotrophic partner means that the growth rate of the microalgae, and their net productivity, may be lower in symbiosis than for free-living algae. This approach results from the considerations of Law and Lewis (1983) and of Douglas and Smith (1989) which emphasise the importance, from the viewpoint of natural selection, of the fates of the genomes of *both* symbionts in a symbiosis. Accordingly, another aim of this paper is to consider the potential for benthic microalgal productivity (in similar habitats) for free-living and for symbiotic examples, i.e. distinguishing net productivity of the microphotobiont from net productivity of the symbiosis.

A third major aim of the paper is to consider the extent to which the chemoorganotrophic component of the symbiosis is 'pre-adapted' to phototrophy, i.e. the extent to which non-symbiotic ancestors have properties which would permit any microalgae which they subsequently associate with to expeditiously acquire photons, inorganic carbon and other inorganic nutrients.

Furthermore, we ask what resource acquisition attributes of the free-living microalgae could be, or are, deputed to the chemoorganotrophic partner, and how any such deputisations are achieved (Raven, 1991a; Raven, Johnston, Handley and McInroy, 1990). Finally, we consider why lichens have never achieved homoiohydry, a mechanism which is apparently limited to terrestrial tracheophytes and which permits greater stature, high productivity and ecological dominance in many terrestrial habitats, by considering the attributes of non-lichenised fungi (Raven, 1986a).

2. Resource Acquisition by Four Categories of Phototrophs: Benthic Microalgae, Benthic Symbioses of Microalgae with Macro-chemoorganotrophs, Benthic Multicellular Macrophytes and Benthic Acellular Macrophytes

As an ecological (but not taxonomic) convenience the term 'microalga' embraces both eukaryotic photolithotrophs and cyanobacteria; furthermore, the term 'benthic' is used for terrestrial as well as aquatic organisms.

Capacity for light absorption

The data in Table 1 show that most phototroph communities can have sufficient chlorophyll *a*, i.e. $\geq$ 500 mg m^{-2}, to absorb more than 95% of the incident photosynthetically active radiation (Anderson, 1967). This is certainly true of free-living terrestrial and marine benthic microalgae, terrestrial and marine algal and higher plant benthic macrophytes, and benthic marine symbioses between microalgae and invertebrates. The only exceptions are, apparently, lichenised fungi with chlorophyte or cyanobacterial symbioses. At the individual organism level, the important point is the area, and chlorophyll (and other pigment) content per unit area, exposed to incident radiation per unit biomass which can process and use photosynthetically active radiation. This is essentially the point made by Porter (1976) who points out that chemoorganotrophic corals have a lower surface area per unit volume than do phototrophic examples.

At least in a vector radiation field it is the *projected* area which determines the capacity to display photosynthetic pigments and absorb photons. In a scalar radiation field it is the 4π equivalent of the projected area which is important. In both sorts of light field any surface area amplifications within the relevant projected area are much more likely to be related to the acquisition of chemical resources, with a large surface area helping to overcome constraints related to fluxes through the lipid bilayer, the number of membrane transporters per unit biomass, or the presence of unstirred layers.

Table 1. Chlorophyll *a* per m^2 substratum of a number of benthic populations or communities

Organism	mg chlorophyll *a* m^{-2}	References
Nostoc commune (terrestrial cyanobacterium)	639–919	Scherer, Chen and Böger (1988)
Parmelia physodes	101–193	
Peltigera canina	34–97	Wilhemsen (1959)
Xanthoria parietina	59	
(terrestrial lichens)		
Terrestrial vascular plant communities	$\leq$ 2000	Talling (1961)
Marine epilithic cyanobacteria (top of littoral)	270–800	
Marine haptophytic *Fucus* (Phaeophyta)	1470	
Marine haptophytic *Laminaria* (Phaeophyta)	400–8000	Raven (1981)
Marine rhizophytic seagrass *Posidonia*	2100	
Marine reef coral *Porites* with *Symbiodinium*	200–2500	

In quantitative terms we can illustrate the argument in the two preceding paragraphs as follows: On a sunny day an unshaded terrestrial microalga or lichen, or a near-surface alga or invertebrate-alga symbiosis in the sea, could be exposed to a vector radiation field of photosynthetically active radiation (400–700 nm) of 100 μmol photon $m^{-2}s^{-1}$. The chlorophyll *a* per substrate area values in Table 1 would permit essentially complete absorption of the incident 400–700 nm radiation (Anderson, 1967; Raven, 1984a). With a photon requirement of 10 mol photons absorbed per mol CO_2 fixed (Raven, 1984a), a CO_2 fixation rate of 100 μmol (m^2 projected area, i.e. substrate area)$^{-1}$ s^{-1} would be permitted. However, the highest possible active influxes of inorganic C across the plasmalemma exposed to air (35 Pa CO_2) or seawater (2 mol m^{-3} inorganic C) is 10 μmol inorganic C m^{-2} s^{-1} (Raven, 1984a). This means that an amplification of total area relative to the projected area by a factor of 10 is needed to permit full use of high incident photon flux densities. For this

reason the discussion of the net photosynthetic rates on a projected area basis (Tables 2–4) will relate mainly to the acquisition of inorganic C and other chemical resources.

Inorganic C supply to terrestrial photolithotrophy

Table 2 considers terrestrial photolithotrophs with an emphasis on microalgae and lichens and organisms of similar life-form to lichens. The net photosynthetic rates of the microalgae *Nostoc, Apatococcus* and *Chlorella* are of a very similar magnitude to those of foliose (*Peltigera*) and crustose (*Xanthoria*) lichens. These photosynthetic rates are clearly not limited by the capacity to absorb light, since the data were obtained at light saturation. Limitation by CO_2 diffusion is more likely to constrain the rate; while diffusion in the gas phase is unlikely to limit photosynthesis unless the gas-phase boundary layer thickness exceeds several mm (possible under still conditions for algae or lichens on soil or tree-trunks: see Nobel, 1991), the diffusion of CO_2 in the aqueous phase could well be limiting for an algal mat, or crustose or foliose lichen, lacking gas spaces between the microscopic algae (Raven, 1984a; Raven et al., 1990). This latter aqueous-phase constraint could be largely eliminated by the presence of extracellular carbonic anhydrase which could, by speeding the extracellular interconversion of CO_2 and HCO_3^-, enlist HCO_3^- as another, and quantitatively dominant, inorganic carbon species diffusing in parallel with CO_2 from the atmosphere-organism interface to the plasmalemma of the microalgae (Raven et al., 1990). While extracellular carbonic anhydrase has been found in the intertidal lichen *Lichina pygmaea* (Raven et al., 1990), data on terrestrial algae and lichens seem to be lacking. However, the occurrence of extracellular carbonic anhydrase is certainly indicated by the characteristics of CO_2 assimilation by mats of *Nostoc commune* (Coxson and Kershaw, 1983a,b; Coxson, Brown and Kershaw, 1983). Furthermore, as with the better-suited aquatic cyanobacteria, the photosynthetic characteristics of terrestrial cyanobacteria, and of 'cyanobacteria-only' terrestrial (and intertidal) lichens, show the operation of a 'CO_2 concentrating mechanism' (Raven et al., 1990; Griffiths, Raven and Maguas, in preparation). This mechanism can operate to increase the CO_2 fixed per unit water lost from terrestrial photolithotrophs, thus increasing the amount of CO_2 which can be fixed per liquid water episode for cyanobacteria-only lichens, or free-living terrestrial cyanobacteria (Raven et al., 1990; Surif and Raven, 1990). Evidence as to the occurrence of a 'CO_2 concentrating mechanism' in free-living terrestrial green algae of the type which occur in lichens, or in lichens in which green algae fix essentially all of the CO_2, thus apparently forgoing the advantage

Table 2. Comparison of net photosynthetic rates of free-living soil-epiphytic terrestrial algae with those of thalloid/crustose liverworts and lichens, and C_3 and C_4 vascular plants, in air; data also given based on primary productivity measurements of (non-crustose!) mosses

Organism	Net photosynthesis at light saturation, 35 Pa CO_2, optimal water content μmol m^{-2} s^{-1} (based on 1 side of thallus, assuming no CO_2 gain from beneath)	Reference
Nostoc commune (free-living cyanobacterium)	0.9 (25°C)[1]	Coxson et al. (1983) Coxson and Kershaw (1983a,b)
Apatococcus lobatus (Chlorophyta)	3.8 (20°C)[2]	Bertsch (1966)
Prasiola stipitata (Chlorophyta)	3.3 (10°C)[2]	Raven and Johnston (1991)
Chlorella pyrenoidosa (Chlorophyta: 'artificial leaf')	0.44 (25°C)[4]	Bidwell (1977)
Peltigera (foliose lichen)	2.1 (15–17°C)[2]	Ried (1960)
Xanthoria mawsonii (crustose lichen)	2.84 (5–10°C)[2]	Kappen (1988)
Cladonia rangiferina (fruticose lichen)	$\leq$ 15.1 (18°C)[6]	Kappen (1988)
Usnea aurantiaco-atra (fruticose lichen)	2.10 (5–15°C)[7]	Kappen (1988)
Monoclea forstori (liverwort)	0.81 (15°C)[2]	Snelgar (1981)
Marchantia foliacea (liverwort)	0.99 (15°C)[2] (1982)	Green and Snelgar
Conocephalum conicum (liverwort)	3.61 (20°C)[2]	Fock, Krotkov and Canvin (1969)
Polytrichum alpestre (moss) and other polar mosses	2.45 (< 20°C)[5]	Bowden (1991); Davis (1981)
Triticum aestivum	34 (14–17°C)[8]	Gifford (1974)
Zea mays	51 (27°C)[8]	Gifford (1974)

[1] From mass-based rate of photosynthesis in the laboratory of natural mats of *Nostoc*, and field measurements of mass/area.
[2] From natural populations measured in laboratory.
[3] From rates of photosynthesis of laboratory (suspension) cultures of algae layered as an 'artificial leaf'.
[4] From rates of photosynthesis of cultured thalli.

[5] From maximum net standing crop in Antarctica, and net photosynthesis at temperature optimum and light saturation on a dry weight basis of a boreal Alpine population. This promiscuous estimate is likely to be an upper limit in view of self-shading and 'standing dead' (Crittenden, 1991).

[6] From maximum standing crop, and net photosynthesis (at temperature optimum and light saturation) on a dry weight basis, of a Maritime Antarctic population. This estimate is likely to be an upper limit in view of self-shading and 'standing dead' (Crittenden, 1991).

[7] From maximum net productivity of 1028 g dw $m^{-2}y^{-1}$, assuming some gross net primary productivity ratio as for moss turf quoted by Davis (1981), *mean* day length of 12 hr for productivity and C = 0.45 dry weight.

[8] From CO_2 uptake rate in a polythene enclosure in the field, higher estimates are found for unenclosed populations in the field using energy – or momentum balance methods (Gifford, 1974).

of increase CO_2 fixation per liquid water episode. However, the capacity of both free-living and lichenised terrestrial green algae to photosynthesise and grow without external liquid water provided the relative humidity of the atmosphere is above a threshold value (Bertsch, 1966; Lange, 1988) may reduce the significance of the (putatively) lowered carbon fixation per liquid water episode.

The net photosynthetic rate per unit substrate area for crustose and foliose lichens resembles that for the thallose subaerial alga *Prasiola stipitata*, and for thallose liverworts (Table 2). Green and Snelgar (1982) found that the net photosynthetic rates of the solid *Monoclea forstori* and of *Marchantia polymorpha*, with intercellular gas spaces, are closely similar, and suggested that the occurrence of such gas spaces did not increase the photosynthetic rate of thalloid liverworts in air levels of CO_2 (Raven, 1977). However, the data on *Conocephalum conicum* (Table 1) shows a higher photosynthetic rate than for the other two liverworts; this difference in rates is unlikely to be entirely due to the different experimental temperatures used. Raven (1977) did not consider the possibility of extracellular carbonic anhydrase in enhancing extracellular inorganic C fluxes within non-aerated tissue of terrestrial macrophytes as an alternative to intercellular gas spaces. However, the computations in Raven et al. (1990) on inorganic C fluxes through the non-aerated thallus of *Lichina pygmaea* and of Nobel (1991) on the gas-phase flux of CO_2 within leaves show that the gas-phase pathway supports a larger CO_2 flux from a gas phase to photosynthetic cells in a bulky organ. Clearly the evidence on water infiltration of the gas spaces of terrestrial lichens shows that water infiltration decreases CO_2 fixation from limiting CO_2 partial pressures in the atmosphere (Griffiths et al., in preparation).

The final comparison among terrestrial organisms involves organisms which are not crustose or foliose, but rather fruticose. The high rate of net photosynthesis entered for *Cladonia rangiferina* (Table 2) is an overestimate of the

substratum – area-based rate of photosynthesis since it assumes that the rate of photosynthesis of growing apices applies to the whole thallus (see discussion by Crittenden, 1991). The other fruticose lichen quoted is *Usnea aurantiacoatra*, with a much lower rate directly measured with the whole thallus. These latter data, and the values (Table 1) for the (fruticose) moss *Polytrichum alpestre*, suggest little increase in substratum-area-based net photosynthesis as a result of the fruticose habit in terrestrial cryptograms. However, the photosynthetic rate of *Mnium ciliare* leaves (total leaf area basis) of 0.675 μmol CO_2 m^{-2} s^{-1} (Nobel, 1977) and the ratio of total leaf area to substratum area for *Mnium hornum* of 36 (Proctor, 1979) suggests the possibility of a substratum area-based rates of net photosynthesis of up to 24 μmol m^{-2} s^{-1} (ignoring self-shading!). The two vascular plants share very high net photosynthetic rates on a substratum area basis which can only be achieved by a combination of several layers of leaves and the presence of intercellular gas spaces in each leaf.

Overall, the available evidence suggests that the rates of net photosynthesis on a substratum area basis for crustose and foliose lichens are not greater than that of algal mats, and that the evidence for increased rates on a substratum area basis resulting from the fruticose habit is also not very convincing. However, the available data-base is small, and more targetted research is needed.

Inorganic C supply to aquatic photolithotrophs

Turning to the aquatic habitat, Table 3 shows that the rates of net photosynthesis by algal mats or crusts are similar to the rates for terrestrial

Table 3. Comparison of rates of net photosynthesis of epilithic/epipsammic microalgae with that of crustose macroalgae in seawater on the basis of projected area

Organism	Net CO_2 fixation at light saturated in seawater at indicated temperature/μmol m^{-2} s^{-1}	Reference
Amphora sp (diatom)	3 (O_2; 25°C[1]	Jensen and Revsbech (1989)
Porolithon onkodes	5 (O_2; ~ 25°C[1]	Chisholm, Collingwood and Gill (1990)
Marine benthic microalgae	0.32[2]	Charpy-Roubard and Sournia (1990)

[1] From measured O_2 evolution rate.
[2] From global estimate of net productivity, assuming 12 hr day and loss of 20% of C fixed in day by respiration at night (assumed respiration high because of self shading); no seasonality, 12 hr light and 12 hr dark.

algal mats and crustose or foliose lichens. Calculations by Raven (1991a) show that the observed rates for aquatic algal mats or crusts require the use of HCO_3^- from the environment; use of CO_2 alone, with no catalysis of extracellular conversion of HCO_3^- to CO_2, could not account for these rates. The third value in Table 3 is lower, as is to be expected in view of its global nature.

Table 4 shows data for non-crustose marine macrophytes. On a substratum area basis the corals have higher rates of net photosynthesis than do macroalgae, although the macroalga cited (*Laminaria longicruris*) lives in much cooler waters than the corals. The rates on a substratum area basis for these organisms are substantially greater than the values for mat-forming and crustose algae in Table 3, consistent with the occurrence of a larger surface area of photosynthetic tissue than of the corresponding substratum area for the non-crustose macrophytes in Table 4 (see Table 4.3 of Raven, 1984a, showing values of 14–16 m^2 thallus or leaf area per m^2 substratum area for 3 aquatic macrophytes, i.e. the acellular Chlorophyte *Caulerpa*, the multicellular Phaeophyte *Laminaria*, and the seagrass *Posidonia*). The net rates of photosynthesis on an organism surface area basis in Table 4 are correspondingly lower than the substratum area-based values in that Table, and are comparable to the substratum area-based (= organism surface area basis) values in Table 3. As with the rates in Table 3 the organism area-based rates in Table 4 demand that the HCO_3^- in seawater is being used; the rates cannot be accounted for by the use of CO_2 without extracellular catalysis of the conversion of HCO_3^- to CO_2.

It is of interest that the two highest organism area-based values in Table 4 are for corals. While the experimental temperature for the corals are higher than those for the algae and the seagrass, it is unlikely that this is the whole explanation for the higher values for the *Symbiodinium* – coelenterate symbiosis than for the acellular macroalga (*Codium*), the multicellular macroalgae *Laminaria, Macrocystis* and *Ulva*, and the seagrass *Phyllospadix*. Raven (1981) and Raven and Richardson (1986) consider as unlikely the possibility that the rates based on external surface area of the corals are over-estimates due to part of the net inorganic carbon influx being across the internal cell layer lining the coelenteron. Further downward adjustments of the organism area-based rate of inorganic carbon assimilation could result from a photosynthetic quotient in excess of 1.0, predominance of lipid biosynthesis, high relative rates of NO_3^- assimilation, and diel organic acid cycling of the type found in Crassulacean Acid Metabolism (Rinkevich and Loya, 1984) could all be involved, but seem unlikely to bring the coral rates of net inorganic C entry down to the macroalgal values, and certainly would not yield lower values.

The involvement of HCO_3^- use in coral photosynthesis as required by the observed rates of net photosynthesis is consistent with the relatively high

Table 4. Comparison of rates of net photosynthesis of non-crustose marine macroalgae and of corals on the basis of projected area (p) and of total organism external surface area (e) for O_2 (O) or inorganic C (C)

Organism	Net photosynthesis at light saturation in seawater at indicated temperature/μmol m^{-2} s^{-1}	Reference
Corals in reef	19 (C; p; 25°C)[1]	Kinsey (1991)
Algal pavement	7.5 (C; p; 25°C)[2]	Kinsey (1991)
Laminaria longicruris (Phaeophyta)	10.5 (C; p; < 20°C)[3]	Mann and Chapman (1975); Table 6.5 of Raven (1984a)
Laminaria digitata (Phaeophyta)	2.7 (O_2; e; 10°C)[4]	Drew (1983)
Macrocystis pyrifera (Phaeophyta)	2.5 (O_2; e; 15°C)[4]	Wheeler (1980)
Ulva rotundata (Chlorophyta)	2.8 (O_2; e; 18°C)[4]	Henley et al. (1991)
Codium fragile (Chlorophyta)	3.4 (O_2; e; 22°C)[4]	Ramus (1978)
Phyllospadix torreyi (Seagrass)	3.0 (O_2; e; 15°C)[4]	Drew (1979)
Acropora palmata (Coral)	4.7 (O_2; e; > 20°C)[5]	Rogers and Solosky (1981)
Stylophora pistillata (Coral)	6.5 (O_2; e; 28°C)[5]	Falkowski and Dubinsky (1981)

[1] From gross primary productivity on a yearly basis, assuming no seasonality, a 12 hr photoperiod, and a balance to within a few per cent of 24 hr gross primary productivity (= gross photosynthesis) and respiration in light and dark.

[2] From gross primary productivity on a yearly basis, assuming no seasonality, a 12 hr photoperiod, and 40% of C gained in gross photosynthesis is lost in respiration in light and dark.

[3] From net primary productivity on a yearly basis, assuming no seasonality (hence an underestimate at times of fastest growth), mean 12 hr photoperiod, C lost at night equals 0.135 of net C fixed in photoperiod.

[4] From net photosynthetic rates quoted in references, converted to basis of both sides of laminar photosynthetic organ. For *Codium fragile*, an acellular macrophyte with cylindrical axes, the net photosynthetic rates relate to total thallus surface area.

[5] From net photosynthetic rates quoted in references on basis of total surface area of coral

inorganic C affinity (low half-saturation concentration for inorganic C) observed by Burris, Porter and Laing (1983) for *Seriatopora hystrix*. For the reasons discussed by Raven (1991a) this high affinity for inorganic carbon, combined with the high area-based rate of assimilation (Table 3) strongly suggests the occurrence of an inorganic carbon concentrating mechanism in photosynthesising corals. However, no measurements of a CO_2 concentration in *Symbiodinium* cells in an illuminated coral which exceeds the CO_2 concentration in the medium have been reported, so that direct evidence on this matter is lacking.

The high area-based rates of net assimilation of exogenous inorganic carbon by corals was related by Raven (1981) to their phagotrophic capacity. The argument used was that the lower area-based photosynthetic rates of haptophytic macroalgae related to their having to assimilate inorganic nitrogen, phosphorus, iron, etc. sources through the same surface as that through which photosynthesis occurred, and that it was the limited availability of elements other than carbon which dictated the surface area per unit biomass. The corals, by their phagotrophic capacity, have alternative supplies of nitrogen, phosphorus and iron in particles which supply relatively more of the organisms needs for elements other than carbon than of carbon itself, since organic carbon serves as an energy source in the dark as well as an element incorporated during growth. Thus, even if the carbon:nitrogen ratio (for example) were the same in the organic part of corals in their food, the coral would be relatively carbon-limited for growth by phagotrophy to the extent that respiration related to growth and to maintenance consumed some of the ingested organic carbon. While corals (unlike some other marine microalga-invertebrate symbioses) have high affinity influx mechanisms in animal ectoderm plasmalemma for phosphate, (probably) ammonium and (possibly) nitrate (see later, and Raven, 1980; Miller and Yellowlees, 1989), phagotrophy appears to be an important source of nitrogen and phosphorus (and iron?) for corals.

Conclusions

Summarising the data discussed here, we see that for the crustose and foliose lichens the net photosynthetic rate on a substrate area basis is in the same range as for mats of terrestrial microalgae. For fruticose lichens, the few measurements available suggest relatively small enhancement of substratum area-based net photosynthetic rates by the (as yet unquantified) enhancement of thallus external surface area per unit substratum area associated with the fruticose habit. For corals, the net photosynthetic rate per unit substrate

area substantially exceeds the rate of net photosynthesis by crustose and mat-forming benthic algae, and also exceeds the rate for erect macroalgae and seagrasses. Here there is a significant influence on the substrate area-based rate of photosynthesis as a result of the (also unquantified) enhancement of coral surface area per unit substrate area.

3. Potential for Multiplication of Microalgae in Lichen or Invertebrate Symbioses Relative to Free-Living Benthic Microalgae

We deal here with the possibilities for increase and dispersal of microalgal DNA in the symbiotic relative to the free-living state (Law and Lewis, 1983; Douglas and Smith, 1989). The general situation is that the specific growth rate of the photobiont in the symbiosis, even under optimal conditions, is lower than that of the photobiont when free-living (Douglas and Smith, 1987). Less generally considered is the impact of symbiosis on the specific growth rate of the chemoorganotrophic partner. A notable exception is the demonstration (Douglas and Smith, 1983; Smith and Douglas, 1987) that green hydra maintained, with feeding, had a lower specific growth rate in the dark when it contained algae than when it did not, although, in a 12 hr light, 12 hr dark regime, the presence of algae had no effect on the specific growth rate which was the same as that of the alga-free hydra in continuous darkness. These data were interpreted as representing a nutritional cost tot he hydra of maintaining its symbionts in continuous darkness (Douglas and Smith, 1983; Smith and Douglas, 1987). The absence of a change in specific growth due to the presence of symbionts in illuminated cultures contrasts with the situation when free-living photolithotrophically growing microalgae are compared with otherwise similar chemoorganotrophic microalgae (Raven, 1986b, 1987). However, a closer analogue of the hydra situation is that of free-living phagotrophic microalgae, where little difference in specific growth rate is often found between growth in light and in darkness for cells cultured in the presence of organic substrates even when dark growth represses plastid differentiation (analagous to the alga-free hydra): Raven (in preparation). Using photosynthetic pigment per unit substrate area as a measure of the number of microalgal cells (Table 1), we find that for the terrestrial lichens and free-living algae the number of algal cells can be higher in algal mats than in lichens on a substrate area basis, while (Table 2) the net photosynthetic rates can be similar for the two sorts of microalgal population. However, taking into account the diversion of photosynthate to the mycobiont and its respiration (Farrar, 1990) the absolute algal growth rate can be higher for the free-living microalgae.

For corals compared with marine microalgal mats, the data in Table 4 show that the area-based mass of chlorophyll (hence microalgal cell number) is higher for the corals, as is the net photosynthetic rate on a substrate area basis (Tables 3 and 4). This appears to show a greater potential for net productivity, and for algal specific growth rate, in the coral microalgae relative to free-living microalgae. However, the diversion of *Symbiodinium* photosynthate to the coral partner, and the high respiratory loss over 24 hr (Hatcher, 1990; Kinsey, 1991), means that free-living microalgae could well have higher absolute productivity per unit area and higher specific growth rates than the symbiotic algae.

The computations above make the implicit assumption that resource supply to the two algal populations (free-living and symbiotic) is identical. However, the free-living microalgal populations cannot generally project themselves above the substratum (an exception being stromatolites, a relatively uncommon life-form among extant cyanobacteria: (Kazmierczak and Kempe, 1990), while this is possible for fruticose lichens and corals. This permits shading of the free-living microalgae by the symbiotic microalgae, with their higher (at least in corals) pigment per unit area of substratum and their position between incident solar radiation and the free-living microalgae. These considerations suggest that the higher stature of a number of microalgal symbioses than of algal mats or crusts can permit a greater specific growth rate of microalgae in benthic symbioses than of free-living benthic microalgae. In comparing the potential of benthic microalgal symbioses with that of multicellular or acellular benthic plants for multiplication of phototroph genomes, it would appear that while the multicellular or acellular benthic plants may produce more copies of the phototroph genome per unit substrate area in a given time, the number of potential propagules of the phototroph may be higher in the symbioses. Thus, although the phototroph biomass is not 'diluted' by chemoorganotroph biomass in the multicellular or acellular macrophytes, the potential for conversion of this biomass (and genomes) into propagules may be lower in the multicellular or acellular macrophytes than in the microalgal symbioses where, potentially, all photobiont cells could be involved in propagation upon (e.g.) expulsion of algae from corals after trauma.

Our conclusions here are that the case for microalgal fitness being increased by symbiosis can only be sustained if the symbiosis expresses its potential for producing larger resource-acquiring structures, thus improving resource acquisition at the expense of lower-growing, non-symbiotic microalgae.

4. To What Extent do the Properties of Non-symbiotic Chemo-organotrophs Relate to Their Subsequent Success in Phototrophic Symbioses With Microalgae?

Lichens

The macroscopic form of lichen thalli is generally a function of the chemoorganotrophic (fungal) partner, i.e. the mycobiont (Hawksworth, 1988). Subaerial non-lichenised hyphal aggregates are generally related to spore dispersal rather than resource acquisition. Especially when such structures are relatively long-lived, there must be a continual flow of water and organic and inorganic nutrients from the hyphae in the nutrient substrate to the subaerial macroscopic structure. Respiration for growth and maintenance of the subaerial body demands O_2 entry from the atmosphere; generally these subaerial structures maintain an intercellular gas space system which facilitates O_2 supply. Raven (1983, 1985) notes that, for a given size and shape of organism, and for a given growth rate, a chemo-organotroph needs a substantially less developed gas-phase ventilation system for O_2 supply than does a photolithotroph for CO_2 supply from the extant atmosphere. This, in turn, permits more water loss from the subaerial structure when it is photolithotrophic; evaporative water loss is energised by the necessary pigmentation (and hence thermal load when in the light) of photolithotrophs. We see that, qualitatively, non-lichenised fungi produce a ventilation system in fruiting bodies and supply water to subaerial evaporating surfaces; however, a similarly-sized lichen growing at a similar rate would need a better-developed ventilation system if CO_2 is to be supplied, and a higher-conductance liquid water transport pathway if evaporative loss is to be replaced. Even allowing for the lower specific growth rate of lichens than of fruit bodies these quantitative requirements remain.

Fungi thus have the potential to produce a ventilated subaerial structure with a liquid water supply. Quantitative adjustments are needed to accommodate photosynthetic CO_2 supply and water loss when the organism is lichenised. The occurrence of photosynthetic cells in a ventilated subaerial structure not only permits the possibility of higher net photosynthetic rates per unit substrate areas than for free-living macroalgae, but also parallels some of the essential features of homoiohydry in vascular land plants (Raven, 1977, 1984b, 1985, 1986a). Thus, the subaerial fruiting bodies and lichens have intercellular gas spaces, pores connecting these gas spaces to the atmosphere, some degree of water repellency (run-off of liquid water from the surface) and water resistance (low permeability to water) at the thallus surface, and some capacity to supply liquid water from the substratum to the growing thallus (Raven, 1986a). Looking more widely at the fungi, Raven (1986a) points out

that rapid changes of cell shape related to changes in cell turgor occur in (carnivorous) fungi, reminiscent of the mechanism driving changes in stomatal aperture in vascular land plants (although not as readily reversible), and that some mycorrhizal rhizomorphs have apoplastic 'vessels' conducting liquid water, analogous to the xylem of vascular land plants. Despite these possibilities, no lichens have attained homoiohydry, i.e. none have the potential to restrict gas exchange between the thallus and the atmosphere when water supply falls short of evaporative demand, thus keeping the thallus hydrated at the expense of greatly restricting the capacity for CO_2 fixation during the period of restricted water supply. The maintenance of hydration permits organisms in environments with a fluctuating balance of water supply and evaporative water demand to be desiccation-intolerant in the vegetative state, and (*via* poorly-understood mechanisms) to attain a height in excess of 1–2 m (Raven, 1986a). This restriction to the height of poikilohydric plants in environments with an incompletely assured water supply, and thus in which desiccation-tolerance is needed for vegetative survival, restricts the stature of lichens (universally poikilohydric, usually desiccation-tolerant), and hence their ability to compete with vascular land plants in a wide range of terrestrial habitats and to capitalise quantitatively in their qualitative capacity to attain a greater stature than non-lichenised microalgae.

Having dealt with an (apparent) missed opportunity on the part of lichens, we now examine the extent to which the capacities lichens *can* achieve is related to the pre-symbiotic characteristics of the fungal partner. For rhizophytic (*sensu* Luther, 1949; Raven, 1981) lichens, i.e. those with rhizines penetrating a fine-grained substratum, uptake of inorganic nutrients and water could be important; more generally, even haptophytic (or pleustophytic: Luther, 1949: Raven, 1981; Rogers, 1971) lichens have the fungal partner as the one most likely to make contact with exogenous liquid water and the solutes therein.

Desiccation tolerance, as a frequent (but not invariable) correlate of poikilohydry, seems to be an attribute of free-living fungi which also characterises the fungal partner in many lichens. The desiccation tolerance of free-living terrestrial microalgae accounts (with fungal desiccation tolerance!) for desiccation tolerance of the overall symbiosis. Despite earlier claims, microalgae in lichens are not able to continue photosynthesis at lower ψ_W values (i.e. at a greater extent of desiccation) than are isolated lichen microalgae (Lange, Pfanz, Kilian and Meyer, 1990), so there seems to be no special role for the mycobiont here.

Fungal attributes in the lichen symbiosis for which less precedent is found among the free-living fungi are UV-B screening (i.e. 280–320 nm) and induction of photosynthate release from the photobiont. The presence of UV-B-absorbing pigments in the (fungal) layers of the lichen overlying the photobiont can help

to screen the photobiont from UV-B. The utility of such an arrangement, with a relative absence of screening of the upper fungal layers, implies a lower sensitivity to UV-B of the mycobiont than of the photobiont. Very large differences in sensitivity to UV-B damage are known for a major target molecule, DNA, among quite closely related organisms, with similarly large differences in the capacity for damage repair, so a substantially greater sensitivity of the photobiont to UV-B damage is possible but unproven (Darentz, Cleaver and Mitchell, 1991; Raven, 1991b). Another major UB-B target, plastoquinone (Raven, 1991b) is specific to O_2-evolving phototrophs, but there is no obvious reason why the equally essential respiratory catalyst, ubiquinone, should not be an equally good target. Thus, UV-B damage to quinones involved in catalysis of respiratory or photosynthetic reactions should not distinguish between aerobic chemoorganotrophs and O_2-evolving photolithotrophs. However, in view of the above-mentioned differences in UV-B sensitivity of DNA, there could be differences in UV-B sensitivity between the two quinones. At all events, there is similarity between the UV-B screening in lichens and that in terrestrial vascular plants. In both cases the UV-B screening compounds are located in non-photosynthetic cells (epidermis in vascular land plants; fungal cells of lichens) between the photosynthetic cells and the incident radiation, with the proviso that stomatal guard cells are epidermal yet almost universally have photosynthetic activity, and that some epidermes have photosynthetic cells other than guard cells; however, this applies mainly to shade plants with little UV-B incident on them (Raven, 1972a,b).

Terrestrial free-living microalgae are not without UV-B screening potential despite the absence of a fungal layer over them. Raven (1991b) points out that microalgae of picoplanktonic size (radius ≤ 1.0 μm) cannot produce effective UV-B screening without addition of a sheath of screening material which is much larger than the cell. UV-absorbing pigments have been identified in the sheath of cyanobacterial filaments composed of rather larger than picoplankton-sized cells (Scherer et al., 1988; Garcia-Pichel and Castenholz, 1991); although these pigments do increase absorption of UV-B, their absorption peak, like that of intracellular screening compounds such as mycosporine-like amino acids, is in the UV-A region of the spectrum. While cyanobacterial *mats* show high UV-B absorbance when the screening compounds are present, the cells on the *surface* of the mats are likely to be damaged (Raven, 1991b).

The other aspect of lichen functioning which has no obvious analogue in free-living fungi, but which occurs in other biotrophic fungi, is the induction of the efflux of particular photosynthetic products from the photobiont. Attempts to isolate and characterise chemical factors responsible for this reversible alteration of photobiont metabolism and/or plasma membrane properties have

had limited success, and physical factors have been invoked in the fungus-alga interaction (Douglas and Smith, 1983). Perceptions of the effect of pressure on plant membrane behaviour, implicit in phenomena such as turgor regulation, have been sharpened by the recent demonstration that stretch-sensitive ion channels occur in the plasma membrane of plants, fungi and prokaryotes as well as the metazoans in which they were first identified (Falke, Edwards, Picard and Misler, 1988; Morris, 1990; Zhou, Stumpf, Hoch and Kung, 1991). While the stretch-sensitive channels identified so far transport ions, these could be a means of triggering neutral solute channels, and there could also be neutral solute channels which are stretch-activated but which can clearly not be directly demonstrated by current patch-clamp techniques if the catalysis is of neutral solute uniport rather than of ion symport. Such possibilities should be examined in the context of the lichen symbiosis.

The discussion of the lichen symbiosis in the context of the intrinsic properties of fungi suggests that while the lichen symbiosis has frequently capitalised on the fungal structure to elevate the photobionts above their free-living relatives, no lichen has yet combined features found in various fungi to become homoiohydric.

Alga-invertebrate symbioses

Dealing especially with corals and tridacnids, we first consider the relation of the structure and function of non-symbiotic relatives of symbiotic corals and tridacnids to the functioning of symbiotic associations.

We have already seen that the retention of phagotrophy by many invertebrates containing symbiotic microalgae, by supplying particulate nutrients, may account for the larger area-based net photosynthetic rate in corals than in macroalgae. This hypothesis requires implicitly that the area required for uptake of particulate N, P, etc. is less than that needed for uptake of these elements in solution. Alternatively, a different area of membrane is used for inorganic C (and soluble N and P source) uptake (external surface) and for particle (tentacles, coelenteron) acquisition.

For those tridacnid clams which are symbiotic with *Symbiodinium* sp. no data seem to be available on the area of the gill, with its gas exchange and particle filtering function, as compared to similar non-symbiotic clams (including some members of the genus *Tridacna*), except for the observation that gill areas in the symbiotic tridacnids 'appear to be relatively small' (Mangum and Johansen, 1982), although the gill of the symbiotic *Tridacna derasa* appears to be modified for increased filtration efficiency (Purchon, 1977). Mangum and Johansen (1982) provided much-needed quantitative data related to particle

filtration and gas exchange by measuring the ventilation rate, and the dark O_2 uptake rate and fractional O_2 extraction $[([O_2]_{inspired}-[O_2]_{expired})/[O_2]_{inspired}]$ in the symbiotic *Tridacna squamosa* for comparison with non-symbiotic but otherwise similar bivalves. While the dark respiratory O_2 uptake rate is unexceptional by comparison with the value for non-symbiotic bivalves, the ventilation rate in the dark is much lower and the fractional O_2 extraction is correspondingly higher (Shick, 1990). While the ventilation rate in the light is lower than in the dark, there is still significant ventilation in the light.

In reinforcing and amplifying the comments on the significance of ventilation rates in symbiotic tridacnids made by Mangum and Johansen (1982), we note that the net rate of inorganic C uptake and O_2 loss during very slow ventilation in the light is at least as great as the dark respiratory rates of O_2 and CO_2 release with rather faster ventilation when rates are expressed on a clam mass basis. While no measurements of inorganic C depletion between inhaled and exhaled water are available for comparison with the 41% extraction of O_2 in the dark, it is clear that the inorganic C entry must involve HCO_3^- as well as (or instead of) CO_2. The argument is that the biomass-based rate of inorganic C uptake in the light is at least as great as that of O_2 uptake in the dark; since there is removal of almost 100 mmol m^{-3} O_2 from water during ventilation in the dark, we would expect more than 100 mmol m^{-3} inorganic C to be removed from water during slower ventilation in the dark, yet the free CO_2 concentration in the inspired water is unlikely to exceed 15 mmol m^{-3} (see Table 5.1 of Raven, 1984a). Such HCO_3^- use is consistent with the suggestion, based on the large excess of O_2 in blood relative to seawater, that a CO_2 concentrating mechanism occurs in symbiotic tridacnids (Raven, 1991a,c). The direct evidence for such a mechanism in a chemolithotrophic (symbiotic) vestimentiferan is not, apparently, available for symbiotic photolithotrophs involving invertebrates (Raven, 1991a,c).

The other uses of ventilation relate to particle extraction and acquisition of soluble forms of such nutrient elements as N, P and Fe. We note that the need for phagotrophy in symbiotic tridacnids may be lower than that needed for non-symbiotic organisms growing at the same rate, since (by arguments made earlier) phototrophic C acquisition means that phagotrophy does not provide an excess of other elements relative to C, in agreement with the absence of NH_4^+ excretion in symbiotic tridacnids (but not in non-symbiotic tridacnids). Indeed, symbiotic tridacnids can apparently extract NH_4^+ and NO_3^- from seawater (Wilkerson and Muscatine, 1986; Miller and Yellowlees, 1989), thus further increasing N availability to the clam. Those considerations help to explain the lower ventilation rates in symbiotic than non-symbiotic tridacnids.

Other uses of phagotrophy in symbiotic photolithotrophic symbioses involving invertebrates include reinfection after sexual reproduction in the majority of cases in which transovarian transfer of symbionts does not occur (Fitt and Trench, 1981; Law and Lewis, 1983), and (possibly) as a means of replacing a population of symbionts more appropriate to some change in the environment (Dustan, 1979). Such changes in the genetics of the photosynthetic entity is not possible in a 'real' plant with integrated endosymbionts (plastids) with little or no genetic diversity within a plant genotype, although the phagotrophic symbiotic photolithotroph may fall victim to not finding the appropriate symbionts in the free-living pool.

Turning now to invertebrate characteristics in microalga-invertebrate symbioses which are less predictable from the characteristics of related non-symbiotic invertebrates, we find examples in biochemical and behavioural responses to the flux density of ultraviolet (damaging UV-B, potentially useful UV-A) and photosynthetically active radiation, and in the occurrence of high-affinity transport systems for such solutes as HCO_3^-, NH_4^+ and $H_2PO_4^-$.

Ultraviolet responsiveness has been reported for photo-receptors of symbiotic tridacnids in contrast to the situation in most molluscs (Wilkens, 1984). Responsiveness to ultraviolet and photosynthetically active radiation is translated into behaviour which maximises absorption of photosynthetically active radiation under light-limiting conditions and avoids (at least in part) excessive, photoinhibitory, photon flux densities, using the animal's nervous and muscular apparatus (Wilkens, 1986; Raven, 1989).

Screening of UV radiation in marine invertebrates and free-living algae, and also in symbiotic algae in invertebrates, frequently involves mycosporine-like amino acids (Raven, 1991b; Shick, Lesser and Stochaj, 1991). It is not likely that the mycosporine-like compounds in symbiotic invertebrates can be regarded as unpredictable from a knowledge of non-symbiotic invertebrates, so they will not be further considered here; for similar reasons, nor will increases in the animal tissues in the levels of metabolites and enzymes which deal with toxic oxygen species related to the enhanced O_2 levels generated by symbiotic photosynthesis (Shick, Lesser and Stochaj, 1991; Raven, 1992), or the increased carbonic anhydrase activity in symbiotic invertebrates presumably functioning in inorganic carbon transport for photosynthesis (Weis, 1991; Weis, Smith and Muscatine, 1989).

An intriguing recent finding relates to the *use* of UV-A radiation by the deep-growing (–95 m – –145 m) symbiotic scleractinean coral *Leptosiris fragilis* in the Gulf of Aquaba where there is a substantial photon flux density of UV-A. The coral tissues contain UV-A absorbing pigments which fluoresce in the photosynthetically active radiation (400–700 nm) range; this fluoresced

radiation is absorbed by photosynthetic pigments and is used in photosynthesis (Schlichter and Fricke, 1990). This mechanism may help account for the exceptionally low *in situ* photon flux density required for compensation of respiration by photosynthesis, i.e. 1.5–2.0 μmol photon m^{-2} s^{-1} (Schlichter and Fricke, 1990). A further coral tissue characteristic which can help explain the low compensation photon flux density is the presence of pigment granules in chromatophores which occur among the algal cells; the chromatophores scatter light and could enhance absorption by *Symbiodinium*. These two features of *Leptosiris fragilis* are animal features specifically related to the photobiont's performance; while the chromatophores could be considered as predictable, the UV-A absorption and efficient re-emission at > 400 nm is less predictable.

The other 'unpredictable' animal attribute is that of the occurrence of transporters in the plasma membrane of the invertebrate which lead to a higher internal concentration than that in the medium of such essential (for photolithotrophy) solutes as CO_2, NO_3^-, NH_4^+ and $H_2PO_4^-$. This assertion as to the occurrence of such transporters needs some comment.

The evidence for a higher internal than external concentration of CO_2 during steady state net photosynthesis is, as indicated earlier, indirect for both symbiotic corals and symbiotic tridacnids. However, it is difficult to avoid the conclusion that such accumulation of CO_2 relative to, and obtained from, the external medium does occur. Furthermore, the symbiotic corals and symbiotic tridacnids have, as is also indicated above, the capacity to use external HCO_3^-, and the evidence for a higher internal than external CO_2 concentration in the light means that such use cannot be just the catalysis of external conversion of HCO_3^- to CO_2, but must involve active transport of HCO_3^- or (possibly) of CO_2 produced by external conversion of HCO_3^- to CO_2 (Raven, 1991a). Furthermore, it is difficult to rationalise the available data in terms of CO_2 accumulation occurring solely at the membranes of the photobiont; such processes are needed at the plasmalemma.

Miller and Yellowlees (1989) have produced an excellent critique of the diffusion/depletion hypothesis of inorganic nutrient, and particularly N, acquisition with particular reference to symbiotic corals. They point out that electrical considerations at the coral plasma membrane (Raven, 1980) mean that the diffusive entry of the anions $H_2PO_4^-$ (or (HPO_4^{2-} or NO_3^-) is extremely unlikely. However, the data (reviewed by Miller and Yellowlees, 1989) on NO_3^- and $H_2PO_4^-/HPO_4^{2-}$ uptake by corals (and symbiotic tridacnids: Wilkerson and Trench, 1985) have essentially all been obtained by measuring depletion from the medium. This procedure does not rule out participation of surface-associated microorganisms (a criticism which does not apply to the CO_2-HCO_3^- data discussed above for several reasons, i.e. use of ^{14}C in the work

of Burris, Porter and Laing (1983), the measurement of internal O_2 concentrations in the work of Mangum and Johansen (1982) and of Shick and Dykens (1985) (Raven, 1991a,c), and the magnitude of the net influxes of inorganic C (Raven, 1991a and above)). However, if these high-affinity transporters *are* associated with the invertebrate, they represent a radical difference from the usual (non-symbiotic) behaviour of invertebrates, where NO_3^- is not a quantitatively important metabolite, while phosphate is obtained from particulate food and any net flux across the plasma membrane represents excretion.

For NH_3/NH_4^+, Miller and Yellowlees (1989) are content (their Fig. 3) with a diffusive entry of NH_3 at the coral plasma membrane, a mechanism which is consistent with the diffusion/depletion hypothesis. Using the cytoplasmic $NH_4^+ + NH_3$ concentrations for cnidarians quoted on p. 116 of Miller and Yellowlees (1989), i.e. 5–50 mmol m^{-3}, and the cytoplasmic pH they (very reasonably) assume, i.e. 7.4, the NH_3 concentration in the cytosol is (using the pKa value quoted by Miller and Yellowlees, 1989, i.e. 9.25) 70–700 μmol m^{-3}. With external $NH_3 + NH_4^+$ of 10 mmol m^{-3} in the medium (perhaps rather high for a tropical reef) we compute a medium concentration of 820 μmol m^{-3} NH_3.

We can use these values of external and internal NH_3 concentration to compute the net NH_3 influx using the equation (1)

$$J = P \times (C_0 - C_i) \tag{1}$$

where J = influx of NH_3 (mol m^{-2} s^{-1})
P = permeability coefficient (m s^{-1})
C_0 = external concentration (mol m^{-3})
C_i = internal concentration (mol m^{-3})

Using P for NH_3 of 6.4×10^{-6} (Ritchie, 1987), J = 0.77–4.8 nmol m^{-2} depending on whether the upper or lower limits on NH_3 concentration in the cytosol are used. These values for $(NH_4^+ + NH_3)$ influx 1/1000 or less of the rates of net C assimilation in the light by symbiotic corals (Table 3), so (even allowing for the possibility that $(NH_4^+ + NH_3)$ influx could occur over the whole 24 hr while net C fixation can only occur in the light) this mode of $(NH_4^+ + NH_3)$ entry could supply very little N relative to the net photosynthetic C fixation. However, we must relate the N uptake to *net* productivity, i.e. allow for the very large fraction of the net inorganic C fixed in the photoperiod which is lost in dark respiration (Hatcher, 1990; Kinsey, 1991). With only 1% of the net photosynthetic C fixation retained at the end of the succeeding dark period,

NH_3 entry would yield C:N assimilation rates over 24 hr of 9.8–61. While the lower of these values would provide reasonable C:N ratio in the coral, we note that the net productivity may be underestimated and the ($NH_4^+ + NH_3$) concentration in reef waters may be overestimated, leading to higher C:N assimilation ratios. Thus, while NH_3 entry could provide significant amounts of N to corals, some of the data on ($NH_4^+ + NH_3$) disappearance from the medium around symbiotic corals and tridacnids appears to need a NH_4^+ transporter (Muscatine and D'Ella, 1978; Muscatine, Masuda and Burnap, 1979; Wilkerson and Trench, 1986).

These considerations on inorganic C, N and P acquisition by symbiotic invertebrates clearly show that more work is needed to determine if all of the transporters postulated (CO_2/HCO_3^-, NH_4^+, NO_3^-, $H_2PO_4^-/HPO_4^{2-}$) occur at the plasma membrane of invertebrates with photobionts. However, on the reasonable assumption that at least the CO_2/HCO_3^- transporter is a *bona fide* component of the invertebrate plasma membrane, and leads to accumulation of HCO_3^- and CO_2 in the invertebrate cytosol, we note that this is not possible with any of the known metazoan HCO_3^- transport systems; only a thermodynamically downhill HCO_3^- entry could occur (Grassl, 1991). Similarly, any high-affinity transporter for NH_4^+, NO_3^+ or $H_2PO_4^-/HPO_4^{2-}$ which occurs in the invertebrate host, and which leads to a higher concentration of the transported solute in the cytoplasm than in the medium, has no obvious analogue in the plasma membrane of non-symbiotic animals.

One hypothesis as to the occurrence of such transporters in the invertebrate plasmalemma is that they are coded by symbiont genes transferred from the symbiont to the host nucleus. Since such transporters at the plasma membrane are needed both in symbiosis and when free-living (Miller and Yellowlees, 1989; Raven, 1980, 1984a), copies of the transporter genes must be retained by the photobiont. Such a possibility is testable, and has implications for the evolution of organelles *sensu* Douglas (in press, quoted by Smith, 1991).

5. Conclusions

The data and concepts discussed in this paper suggest that symbiosis with benthic chemoorganotrophs can enhance the rate of energy and inorganic carbon acquisition per unit substrate area relative to the free-living benthic microalgae under natural conditions. This enhancement is more pronounced for marine invertebrate symbioses than for lichens. This symbiotic enhancement of resource acquisition can lead to enhanced microalgal genome production analagous to what can occur in macrophytes derived asymbiotically by acellular or multicellular elaboration of an algal unicell. While some of the attributes

of the symbioses are predictable from the properties of the asymbiotic ancestors of the chemoorganotrophic partner, others (e.g. high-affinity systems for uptake of inorganic C, N and P by marine invertebrate symbioses) are not.

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