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# Characteristics of *Prochloron*/Ascidian Symbioses II. Photosynthesis-Irradiance Relationships and Carbon Balance of Associations from Palau, Micronesia

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**Abstract**

Photosynthesis-irradiance (P-I) relationships, respiration, and photosynthesis/respiration ratios (P:R) are compared for six species of *Prochloron*/ascidian symbioses in Palau, West Caroline Islands: *Lissoclinum patella, Lissoclinum punctatum, Lissoclinum voeltzkowi, Diplosoma similis, Diplosoma virens*, and *Trididemnum cyclops*. The colonies were collected in a shallow coral lagoon in fully exposed, high photon-flux environments and in shaded environments. For the *L. patella* symbiosis, we determined the $Q_{10}$ values for photosynthesis and respiration between 15 and 45°C and the fractional contribution of *Prochloron* carbon production to ascidian respiration demand. The P-I relationships of the colonies demonstrated photo-adaptation features similar to that of the freshly isolated symbiont and reflected adaptation to the photosynthetic quantum fluxes inside the animal rather than total quantum fluxes incident on the colonies. All of the colonies except *L. voeltzkowi* showed daily $P_{net}$:$R_{colony}$ ratios greater than 1.0 when determined by using daily periods of saturating and compensating light. When the daily $P_{net}$:$R_{animal}$ ratios and the contribution of symbiont carbon to host respiration were determined for high- and low-light colonies of *L. patella*, the ratios were between 1.5–2.8, while the carbon contribution from the symbiont to the host was between 30–56%. The log transformation of photosynthetic rate vs. temperature relationships yielded a discontinuity at the ambient growth temperature (30°C); the

$Q_{10}$ below ambient was 3.52 while it was 1.62 above ambient temperatures. The $Q_{10}$ for colony respiration was 1.97 over the entire temperature range (15–45°C). The estimated production rates of these symbioses were 13.1 $\mu$g C $dm^{-2}$ $d^{-1}$. Collectively the results indicate that *Prochloron* can make significant contributions to the nutrition of its hosts, that *Prochloron*/ascidian symbioses make substantial contributions to benthic production in coral reef systems and that the low temperature sensitivity of symbiont photosynthesis probably restricts the distribution of the symbioses to warm water.

Keywords: *Prochloron*; ascidian; photosynthesis; respiration; symbiosis; temperature responses; $Q_{10}$; carbon balance; thermal distribution; prokaryote; benthic primary production; coral reef

Abbreviations: P-I, photosynthesis vs. irradiance, P:R, photosynthesis/respiration ratio; PSU, photosynthetic unit; PAR, photosynthetically active radiation; $I_c$, photsynthetic compensation point, $I_k$, photosynthetic light saturation; chl, chlorophyll; CPAR, carbon contribution from *Prochloron* to animal respiration; $H_{SAT}$, daily light period $\geq$ light saturation; $H_{comp}$, daily light period $\geq$ light compensation.

## 1. Introduction

At least 30 species of ascidians (Protochordata), most of them belonging to the family Didemnidae, have photosynthetic symbionts (Kott, 1982; Cox, 1986). Of these the majority are associated with the green prokaryotic alga *Prochloron* sp., a representative of the division Prochlorophyta (Lewin, 1975, 1977), whereas a few possess cyanobacteria in the genus *Synechocystis* (Lafargue and Duclaux, 1979; Cox, 1986). Those colonial didemnids that contain *Prochloron* are found almost exclusively in warm tropical waters, in habitats ranging from surfaces of coral rubble and rocks, on seagrass leaves and mangrove roots in fully exposed areas to more shaded and protected sites among coral branches (Kott, 1977, 1982; Lewin 1981; Lewin et al., 1983; Ryland et al., 1984). In the Palau Archipelago, West Caroline Islands, *Prochloron*/ascidian symbioses are not only common but can be locally abundant in some areas such as Ascidian Lake and the Kamori Channel off Koror (Hamner, 1982; Lewin et al, 1983).

In Palau, there are at least seven species of didemnids with symbiotic *Prochloron*, the algal symbiont being either embedded in the test or held by mucilaginous material to the cloacal lining (Lewin and Cheng, 1975; Kott, 1982; Cox, 1986). Algal cell densities are sufficiently great (up to $10^7$ cells $cm^{-2}$) to give the colonies a green-grey to deep forest green color. Photosynthesis by isolated *Prochloron* has been shown to be as great as

(Thinh and Griffiths, 1977; Fisher and Trench, 1980; Lewin et al., 1983, 1985; Critchley and Andrews, 1984; Alberte et al., 1986) or greater than that of free-living algae or other eukaryotic symbionts (Muscatine et al., 1981; Falkowski and Dubinsky, 1981; Dustan, 1982; Lee et al., 1982; Chang et al., 1983; Muller-Parker, 1984; Alberte et al., 1986). Consequently, in areas such as the coral lagoon in the Kamori Channel, where *Prochloron*/ascidian symbioses are abundant, they could make a significant contribution to community productivity (see Lewin et al., 1983).

To begin to assess the features of these associations which are important to the nature of their symbiosis and influence their potential contribution to benthic productivity, we characterized and compared the photosynthesis-irradiance (P-I) relationships and daily colony photosynthesis/respiration (P:R) ratios of six common species. Whereas some species (e.g., *Lissoclinum voeltzkowi*) are found only in fully exposed sites in shallow (1–2 m) water, others (e.g., *Diplosoma similis* and *L. punctatum*) are found only in shaded, protected sites, while some species (e.g., *L. patella*, *D. virens* and *Trididemnum cyclops*) inhabit both kinds of light environments in Palau. Since *L. patella* colonies are common to both high and low photon flux environments and since the light adaptation features of *Prochloron* from this symbiosis have already been detailed (Alberte et al., 1986), we used this association to investigate the light adaptation of the symbiotic association and the contribution of symbiont carbon to animal respiration, termed the CPAR (Muscatine et al., 1981). We also measured the $Q_{10}$ of photosynthesis and respiration of colonies of *L. patella* in an effort to define the physiological basis of the restricted global distribution of *Prochloron*/ascidian symbioses to warm waters (Lewin, 1979; Kott, 1982). These results are compared with properties of the isolated algal symbiont and with published data on other symbiotic systems.

## 2. Materials and Methods

### *Habitats and colony collection*

Colonies of the colonial didemnid ascidians *Lissoclinum patella, L. punctatum, Trididemnum cyclops, Diplosoma similis* and *D. virens* were collected in a shallow (1–2 m) coral lagoon in Kamori Channel, Malakal Island, Palau, West Caroline Islands (Lat. 7°25'N; Long. 134°30'E) and transported in seawater to the Micronesia Mariculture Demonstration Center (MMDC) laboratory, where they were held in large tanks with running seawater for a maximum of 24 hr before experimentation.

*Lissoclinum patella* colonies (ca. 5–10 cm in diameter or length, usually found growing on patches of benthic macrophytes such as *Halimeda* sp.) were collected from fully exposed sites near the middle of the channel and from shaded sites which never received photon flux levels greater than ca. 450 $\mu$E $m^{-2}s^{-1}$ (see below; Alberte et al., 1986). Colonies of *L.voeltzkowi* (ca. 1 cm diameter or length) were collected from the tips of leaves of the seagrass *Enhalus* sp., on which they were fairly common but showed patchy distribution. We deliberately selected these populations because they were not shaded by the seagrass canopy or by coastal vegetation. Colonies of *D. virens* (1–1.5 cm diameter) were collected from extensive populations growing on the upper surfaces of rocks and coral rubble in shallow, fully exposed portions of the channel. These populations were frequently fully emergent during low tides, which were near midday during our study period.

Colonies of *T. cyclops, L. punctatum* and *D. similis* (0.5–1.5 cm diameter or length) were collected from the seaward portion of the channel at the margins of a 15 m-deep reef edge, colonies of *T. cyclops* were found in exposed areas on the surfaces of coral rubble and living coral heads, while *L. punctatum* and *D. similis* were found only in shaded environments on the undersides of coral branches.

All collections and experiments were conducted in March 1983, during a period of no rainfall and cloudless skies. Ambient water temperatures at the collection sites were 28–30°C and salinity was 34 $^{o}/_{oo}$.

*Field light measurements and daily irradiance characteristics*

All photon flux measurements were made with a hand-held submersible $4\pi$ quantum PAR (photosynthetically active radiation) sensor (Biospherical Instruments, San Diego, CA; model QSL-140). Daily irradiance curves were generated from hourly measurements made at the sea surface and at a series of depths on at least 5 different days at the exposed and shaded sites. The daily periods of light-saturated photosynthesis, termed $H_{sat}$, and of light compensation, termed $H_{comp}$, were determined from the daily irradiance curves and photosynthetic light saturation and compensation values for the various species (Dennison and Alberte, 1982, 1985).

Reduced photon flux environments similar to those measured in the field sites were created in a flowing seawater tank by using neutral-density screening (Alberte et al., 1986). In shaded conditions the photon flux levels were kept below 450 $\mu$E $m^{-2}s^{-1}$ while high-light conditions were at ambient PAR levels. These light environments were used to hold colonies from shaded

habitats and to maintain discoid (0.79 $cm^2$, 0.6–1.0 cm thick) plugs removed with a cork borer from large *L. patella* colonies. The discs had been kept in running seawater for 5–7 days until the cut edges healed and the plugs were suitable for oxygen exchange measurements (see below).

*Photosynthesis-irradiance relationships*

Photosynthesis and dark respiration rates were determined on whole intact colonies of all species except *L. patella*, colonies of which were too large for the electrode chamber. Colonies were placed on nylon screen in the electrode chamber, with their upper surface towards the light source. The electrode chamber was filled (3 ml) with Millipore-filtered (0.22 $\mu$m) seawater containing 4 mM sodium bicarbonate. Oxygen exchange was measured in a temperature-controlled Clark-type oxygen electrode (Rank Bros., Cambridge, England). For P-I experiments, a range of photon fluxes was obtained by using neutral-density screening. The light level required to saturate photosynthesis, $I_k$, was determined from the intersection of the regression line of the initial slope with a horizontal line through $P_{max}$, while the light level required for photosynthetic compensation, $I_c$, was determined from the intersection of the initial slope with the x-axis. Dark-respiration rates were measured immediately after exposure to each light level. All oxygen exchange measurements were made in triplicate. Details of methods are given by Alberte et al. (1986).

*$Q_{10}$ determinations for photosynthesis and respiration*

Before being used, healed plugs from *L. patella* from exposed sites were pre-treated for 30 min at each experimental temperature (15, 20, 25, 30, 35, 40 and 45°C, ±0.5°C). Oxygen exchange rates were determined in triplicate, as above, except that the electrode chamber was maintained at the same temperature as the pre-treatment temperature. Dark-respiration rates were determined first (5–10 min) followed by measurements of $P_{max}$ at saturating photon flux density (1350 $\mu E\ m^{-2}s^{-1}$). The $Q_{10}$ values for net photosynthesis and respiration were determined over the 15 to 45°C temperature range from the log transforms of rate vs.temperature plots, as described by Berry and Raison (1979).

*Pigment, weight and CPAR determinations*

Chlorophyll (chl $a + b$) contents of colonies were determined on the same samples as were used for oxygen exchange measurements. Colonies were rinsed in fresh water, and then immersed in 100% acetone and stored in the dark at 15°C for 24–36 hr. In most cases the chlorophyll was fully extracted from the colonies without need for homogenization. In some cases the colonies were cut into pieces and further extracted in 80% (v/v) aqueous acetone containing a trace of $MgCO_3$ to reduce pheophytinization. All acetone extracts were brought to 80% (v/v) before spectrophotometric determination of total chlorophyll (chl $a + b$) by using the equations of Arnon (1949).

Fresh and dry weights of colonies were determined for all species. Plugs of *L. patella* were squeezed to remove almost all the algal cells, and fresh and dry weights of the host tissue and of the isolated *Prochloron* cells were determined separately. We estimated the fractional contribution of carbon from *Prochloron* which supports the respiratory demand of its host (*L. patella*) by determining the contribution of host respiration to colony respiration and the carbon translocation rate from the symbiont to the host. Carbon release by *Prochloron* cells isolated from *L. patella* was determined previously to be 20% of the total carbon fixed (Alberte et al., 1986). To estimate the proportion of colony respiration attributable to the host, we deducted dark respiration rates of isolated *Prochloron* cells from *L. patella* from the intact colony dark respiration rates and expressed them on the bases of chlorophyll, area of host, and weights of host and alga. Though in a previous report (Muller-Parker, 1984) isolated symbiont respiration rates were greater (6–8 times) than *in situ* rates, we find isolated *Prochloron* respiration rates to be so low (typical of cyanobacteria) it is unlikely that *in situ* respiration is much lower. Although the biomass of the animal (including test material) exceeds that of the algal cells, we calculated a *Prochloron:L. patella* respiration ratio of 0.50.

The formulations of Muscatine et al. (1981) were used to determine CPAR, where $CPAR = (P_p \times T)/(R_a)$. $P_p$ is the daily net photosynthesis of the symbiont (determined by using $H_{sat}$ and $H_{comp}$ for daily photosynthetic periods), T is the translocation rate, and $R_a$ is the 24 hr animal (host) respiration rate (determined on the assumption that it is constant throughout the daily 24 hr period). Our method of obtaining daily net photosynthesis and respiration was based on short-term measurements of $P_{max}$ rather than on integrated photosynthesis or respiration over 24-hr periods (see Dennison and Alberte, 1985). Since our treatment equally overestimates both daily net photosyn-

thesis and respiration, the effects on the ratios of these features are probably minor. Colony P:R ratios were determined by using colony daily net photosynthesis and either colony or animal (host) 24-hr respiration. For these calculations, oxygen exchange data were converted to equivalent carbon units by using the ratio of $gC/gO_2 = 0.375$. We assumed for the purposes of these calculations that for both the alga and the animal the RQ values were 1.0.

## 3. Results

*Field light environments*

The daily light regimes for exposed and shaded habitats in the Kamori Channel were described previously (Alberte et al., 1986) and are shown in Fig. 1. In the exposed sites, maximum daily photon flux levels reached about 2300 $\mu$E $m^{-2}s^{-1}$, while in the shaded sites flux levels never exceeded 450 $\mu$E $m^{-2}s^{-1}$. The maximum daily quantum fluxes were 73.0 and 10.5 $\mu$E $m^{-2}s^{-1}$ for the exposed and shaded sites, respectively. We determined the daily period of saturated photosynthesis ($H_{sat}$) and photosynthetic compensation ($H_{comp}$) from these curves by using the values of $I_k$ and $I_c$, respectively, obtained from the P-I relationships of each species.

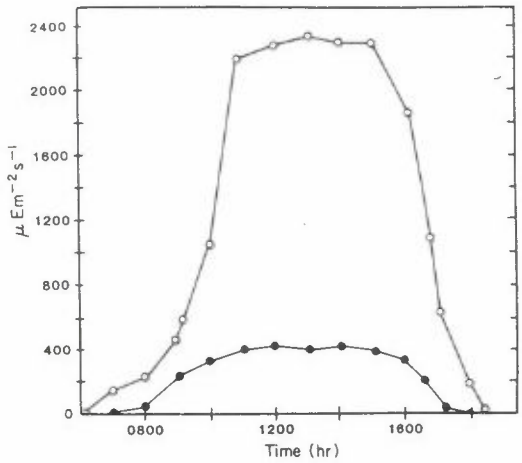


Figure 1. Daily patterns of irradiance (PAR, 400–700 nm) in exposed (open circles) and shaded (closed circles) sites in the Kamori Channel, Palau, measured in March 1983. Each point represents the average 5 measurements on each of 5 different days (N per point = 15–20) under cloudless skies. Photon flux densities were measured with a $4\pi$ PAR sensor.

*Photosynthesis vs. irradiance relationships*

Expressed on a chlorophyll basis, the P-I curves for *L. patella* colonies collected from shaded and exposed habitats and maintained under similar conditions in seawater tanks are shown in Fig. 2A. Colonies from exposed, high-photon flux environments show a higher $P_{max}$ and a shallower initial slope than colonies from shaded sites. The $P_{max}$ and respiration values obtained here for *L. patella* colonies are almost as great as those reported by Pardy (1984). The light requirement for photosynthetic saturation ($I_k$) is 32% greater for the high-light colonies than for the low-light ones, while the photon flux level required to reach photosynthetic compensation, $I_c$ is 40% greater (Table 1). These features are similar to those observed for *Prochloron* cells isolated form *L. patella* (Alberte et al., 1986), and may be taken to indicate photoadaptation. There is a notable difference between the colony respiration rates and those of the symbiont alone. Respiration rates of high-light colonies were more than double those of low-light colonies (Table 1), while isolated symbionts from low- and high-light colonies showed a 4-fold difference (Alberte et al., 1986), suggesting influences of the host on oxygen exchange rates in the symbiont.

Comparisons from the P-I curves of the other *Prochloron*/ascidian symbioses (Fig. 2, A and B) indicate two general features. First, colonies and species collected from exposed habitats (e.g., *L. voeltzkowi, D. virens, T. cyclops* and high-light *L. patella*) all show shallower initial slopes of the P-I curves and higher light compensation and saturation levels than colonies from shaded habitats. Two low-light associations, *D. similis* and *L. punctatus*, show significant photoinhibition at high fluence rates, but colonies of *L. patella* from somewhat similar light environments show no such inhibition of photosynthesis even at 2100 $\mu$E $m^{-2}s^{-1}$ (Fig. 2, A and B). *D. similis* shows severe photoinhibition at flux levels above ca. 600 $\mu$E $m^{-2}s^{-1}$ (Fig. 2B), with a 40% reduction in photosynthetic rate at light levels comparable to those found in exposed sites (200 $\mu$E $m^{-2}s^{-1}$). *L. punctatum* colonies show a similar response to photon flux levels above about 1200 $\mu$E $m^{-2}s^{-1}$ (Fig. 2A). In their natural habitats, *L. punctatum* and *D. similis* colonies probably rarely experience photon fluxes exceeding ca. 200 $\mu$E $m^{-2}s^{-1}$ and, therefore, could be expected to show greater sensitivity to high fluence rates than the low-light colonies of *L. patella*, which routinely experienced flux levels of about 400 $\mu$E $m^{-2}s^{-1}$.

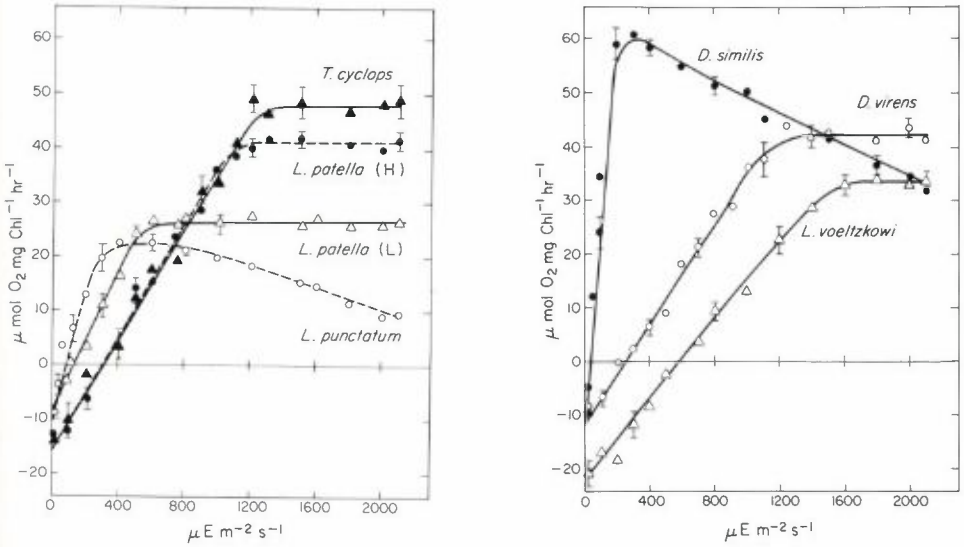


Figure 2. Photosynthesis-irradiance relationships of *Prochloron*/ ascidian symbioses collected in Palau during March 1983. Oxygen exchange measurements were made on whole colonies of each species except for *L. patella* in which case 0.79 $cm^2$ plugs were used.
(A) Plugs of *L. patella* were maintained in tanks with running water under high-light (• – – – •) and low-light (Δ———Δ) conditions similar to those experienced by the colonies from which they came. Colonies of *T. cyclops* (▲———▲) were collected from fully exposed sites on living coral heads, while colonies of *L. punctatum* (○– – –○) were collected from shaded sites on the lower surfaces of coral heads. Irradiance characteristics of the sites are shown in Fig. 1.
(B) Colonies of *D. similis* (•———•) were collected from shaded sites on the lower surfaces of coral rubble, while colonies of *D. virens* (○———○) and *L. voeltzkowi* (Δ———Δ) came from fully exposed habitats. The *L. voeltzkowi* was collected from epiphytic populations on seagrass, while *D. virens* was collected from populations on intertidal rocks.

Colonies of *L. patella* from shaded habitats do not show photoinhibition even at 2100 $\mu E\ m^{-2}s^{-1}$. *Prochloron* cells isolated from these colonies, however, show photoinhibition at photon flux levels above 600 $\mu E\ m^{-2}s^{-1}$ (Alberte et al., 1986). In *L. patella* colonies *Prochloron* cells are present in the cloacal cavities where photon flux levels are 60–80% lower than that incident upon the surface of the colonies (Alberte et al., 1986). Thus, even with 2100 $\mu E\ m^{-2}s^{-1}$ incident on the colonies, the flux level inside the cloaca would be less than ca. 800 $\mu E\ m^{-2}s^{-1}$ and, consequently, photoinhibition would not be expected. Therefore, the degree of light attenuation by the host tissue is

an important feature influencing the P-I responses of *Prochloron in hospite* in different host species. This notion is further verified by the observation that the estimated *in hospite* daily photosynthetic quantum flux levels (total quantum flux $\leq I_k$) for high and low-light colonies of *L. patella* differ by about 30% (32.8 E $m^{-2}d^{-1}$ for exposed colonies and 24.0 E $m^{-2}d^{-1}$ for shaded colonies). A difference of similar magnitude is observed in $P_{max}$ and $I_k$ for the two light conditions rather than the 7-fold difference characteristic of the total daily quantum fluxes in the two light environments (Table 1). A similar finding was made on the isolated *Prochloron* cells from high-and low-light colonies (Alberte et al., 1986).

A somewhat surprising finding is that *D. similis* possesses the highest $P_{max}$ value, the steepest slope of the P-I curve and the lowest $I_c$ value of any of the associations examined (Table 1). In fact, the measured $P_{max}$ value of the colonies is nearly as great as that obtained for isolated *Prochloron* cells from *L. patella* (Alberte et al., 1986), and the chl *a*/*b* ratio of *Prochloron* from these colonies, about 2.1, is lower than found in other associations (ca. 3 to 8; Lewin et al., 1983). In addition, the chl:dry wt ratios for *D. similis* colonies are at least triple those of any of the other associations examined. *L. punctatum* shares many P-I features with *D. similis*, except that its $P_{max}$ is considerably lower and is closer to that of low-light *L. patella*. Colonies of *D. similis* and *L. punctatum* are clearly well adapted to low-light regimes, where they are able to maximize light utilization and photosynthetic performance.

Associations from high photon flux environments show high $I_k$ and $I_c$ values and shallow initial slopes of the P-I curves (Table 1). These associations are less effective in utilizing low quantum flux levels than colonies from low-light habitats. In fact, *L. voeltzkowi* requires 25% full sunlight to even reach photosynthetic compensation, and shows photosynthetic saturation only at photon flux levels approaching full sunlight. *D. virens* and *T. cyclops* colonies from similar high-light environments show similar P-I features. A major difference, however, is observed between the respiration of *L. voeltzkowi* and *D. virens* colonies. *L. voeltzkowi* colonies have a respiratory rate more than double that of *D. virens* and almost double that of even high-light *L. patella*. Algal:animal biomass ratios probably contribute to the differences observed. For example, on a dry weight basis the *L. voeltzkowi* colonies examined contain only one-third as much chlorophyll per unit dry weight as *L. patella*.

When instantaneous P:R ratios are determined on the basis of colony net $P_{max}$ and $R_{max}$ (Table 1), all the associations examined show ratios greater than 1.0. An increase in P:R ratio is observed in low-light colonies

Table 1. Photosynthesis-irradiance relationships and net $P_{max}$:$R_{max}$ ratios of *Prochloron*/ascidian associations from Palau. All colonies were collected from exposed habitats except for shaded colonies of *L. patella*, and all colonies of *D. similis* and *L. punctatum*. The light environments for the colonies are shown in Fig. 1. Values are the means of triplicate determinations.

| Host species | $P_{max}$ | R[a] | P:R Ratio | $I_k$ | $I_c$ | Slope[b] |
|---|---|---|---|---|---|---|
| | ($\mu$mol $O_2$ mg $chl^{-1}hr^{-1}$) | | | ($\mu E\ m^{-2}s^{-1}$) | | |
| *Lissoclinum petella* | | | | | | |
| Exposed site | 40.8 | 13.2 | 3.1 | 850 | 300 | 0.038 |
| Shaded site | 26.4 | 7.2 | 3.7 | 575 | 120 | 0.046 |
| *Lissoclinum punctatum* | 22.2 | 9.0 | 2.5 | 175 | 80 | 0.127 |
| *Trididemnum cyclops* | 46.6 | 15.0 | 3.1 | 1225 | 300 | 0.038 |
| *Lissoclinum voeltzkowi* | 33.6 | 21.0 | 1.6 | 1500 | 575 | 0.022 |
| *Diplosoma virens* | 42.0 | 9.6 | 4.4 | 1175 | 250 | 0.036 |
| *Diplosoma similis* | 60.0 | 10.2 | 5.9 | 200 | 30 | 0.300 |

[a] Maximum dark respiration

[b] Initial slopes of the P-I curves are in units of $\mu$mol $O_2$ mg $chl^{-1}hr^{-1}$/ $\mu E\ m^{-2}s^{-1}$.

of *L. patella* compared to high-light ones. *D. similis* colonies from shaded habitats shows a very high P:R ratio of 5.9. There is no consistent pattern, however, of P:R ratios in relation to growth light environment as *L. punctatum* from low light has a P:R ratio considerably smaller than high-light *L. patella* or *D. virens* from fully exposed sites. For *D. virens*, Thinh and Griffiths (1977) reported P:R ratios of 8.5, almost double the value of 4.4 determined here. From Pardy's (1984) data we calculate a colony $P_{max}$:$R_{max}$ ratio of 2.3 for *L. patella*, for which we obtained values of 3.1–3.7 (Table 1).

*Daily photosynthetic periods and daily colony P:R relationships*

In order to obtain estimates of potential carbon balance between host and symbiont, daily net photosynthesis (based on $P_{max}$) was compared to daily colony respiration (based on $R_{max}$) in the different symbioses (Table 2). We determined the $H_{sat}$ and $H_{comp}$ periods for each association on the basis of the daily light regime in the environment where the collection was made, and determined $I_k$ and $I_c$ values from P-I curves for each species (Table 1). The daily periods when light levels yield photosynthetic saturation ($H_{sat}$) ranged from 6 to 11 hr among the species and light conditions examined (Table 2). The $H_{sat}$ range was 6.1 to 7.1 hr for species from exposed sites, and 7.4 to 11.0 hr for colonies from shaded sites (Table 2). The $H_{comp}$ periods ranged from 11.2 to 12.4 hr for low-light colonies and from 7.0 to 10.2 hr for colonies

Table 2. Characteristics of daily light regimes and $P_{net}$:$R_{colony}$ ratios for six different *Prochloron*/ascidian associations from Palau. The daily periods of light levels sufficient to achieve photosynthetic compensation ($H_{comp}$) and saturation ($H_{sat}$) were used to calculate colony P:R values derived from daily net photosynthesis, assuming colony dark respiration rates were constant over a 24 hr period. The minimum and maximum P:R values were calculated by using $H_{sat}$ and $H_{comp}$, respectively, to determine the daily net photosynthesis.

| Host species | $H_{sat}$ (hr) | $H_{comp}$ (hr) | $P_{net}$:$R_{colony}$ Ratio |
|---|---|---|---|
| *Lissoclinum patella* | | | |
| Exposed site | 6.9 | 10.2 | 0.89–1.30 |
| Shaded site | 7.4 | 11.2 | 1.16–1.76 |
| *Lissoclinum punctatum* | 11.0 | 12.2 | 1.13–1.25 |
| *Trididemnum cyclops* | 6.3 | 10.2 | 0.82–1.33 |
| *Lissoclinum voeltzkowi* | 6.1 | 7.0 | 0.41–0.47 |
| *Diplosoma virens* | 7.1 | 10.2 | 1.30–1.86 |
| *Diplosoma similis* | 10.2 | 12.4 | 2.51–3.05 |

from shaded habitats. These differences in daily photosynthetic periods are important in determinations of daily carbon balance by the symbioses.

In a previous study (Alberte et al., 1986) it was shown that daily P:R ratios based on short-term photosynthesis and respiration rates and $H_{sat}$ periods, rather than photoperiods, gave reliable estimates of carbon balance in isolated *Prochloron*. However, caution must be observed in interpretations of P:R ratios in symbiotic associations when carbon translocation or host contributions to colony respiration are not considered (see Muscatine et al., 1981). Therefore, we employed two methods to estimate colony daily P:R ratios. In the first approach, we related daily net photosynthesis, based on $H_{sat}$ and $H_{comp}$ light periods, to colony 24-hr respiration (Table 2) while in the second (see below) we used symbiont net photosynthesis and related it to 24-hr host respiration (Table 3). In all of the associations examined with the exception of *L. voeltzkowi* (which has a P:R ratio of less than 0.5), the minimum P:R ratios obtained suggest that *Prochloron* is potentially capable of producing sufficient organic carbon to meet a portion of the demands of its hosts (assuming adequate carbon translocation; see below).

*Diplosoma similis* exhibits a daily $P_{net}$:$R_{colony}$ ratio greater than 2.0 (Table 2), which is about of half of that obtained for isolated *Prochloron* cells from *L. patella* (daily P:R = 4.6; Alberte et al., 1986). Such a high minimum daily $P_{net}$:$R_{colony}$ ratio indicates that much of the carbon requirement

Table 3. Estimated daily $P_{net}$:$R_{animal}$ ratios and daily contributions of carbon from *Prochloron* to ascidian respiration demand (CPAR) in *Lissoclinum patella* colonies collected from exposed and shaded sites in Palau. Values were derived by using the formulations of Muscatine et al. (1981; see text). The $H_{sat}$ and $H_{comp}$ light periods were used to calculate daily rates of net photosynthesis and the range of values obtained are presented.

| | $H_{sat}$ (hr) | $H_{comp}$ | CPAR (%) | $P_{net}$:$R_{animal}$ Ratio |
|---|---|---|---|---|
| Exposed site | 6.9 | 10.2 | 29.5–43.6 | 1.48–2.18 |
| Shaded site | 7.4 | 11.2 | 36.8–55.6 | 1.84–2.78 |

of the host could be supplied by its photosynthetic symbionts. In *L. patella* the daily $P_{net}$:$R_{colony}$ ratios increased from 1.30 in the high-light colonies to 1.76 in the low-light colonies, when determined using the $H_{comp}$ periods, indicating the symbiont photosynthesis could make a greater contribution to host carbon demand in shaded colonies than in fully exposed colonies.

*Carbon contribution form Prochloron to Lissoclinum patella*

We determined P:R ratios by using rates of daily photosynthesis and animal respiration daily $P_{net}/R_{animal\ (24-hr)}$ for *L. patella* colonies from exposed and shaded sites (Table 3). Again daily $H_{sat}$ and $H_{comp}$ periods of photosynthesis were determined from $P_{max}$ in the natural light regimes. The values were all above 1.0, irrespective of whether we used the minimum daily light periods and whether the colonies were collected from fully exposed or shaded sites. Since carbon release rates of 20% are known for *Prochloron* from *L. patella* (Alberte et al., 1986), these daily $P_{net}$:$R_{animal}$ ratios would suggest that a significant portion of the host carbon demand could be met by symbiont photosynthesis. Using a similar method and assuming a 20% carbon translocation rate, Olson and Porter (1985) obtained a P:R ratio for *Prochloron*/*Didemnum molle* associations of 0.62.

We also determined CPAR values for *L. patella* colonies from exposed and shaded habitats by using the formulations of Muscatine et al. (1981). Based on a 20% carbon translocation rate (Alberte et al., 1986), the contribution of *Prochloron*-produced carbon to host respiration was 29.5% (determined for colonies in exposed sites, when the daily $H_{sat}$ period was 6.9 hr) (Table 3). This would be a minimum value because considerable photosynthesis occurs at light levels below saturation and because carbon translocation rates are often higher *in situ* than in isolated symbionts (see Muscatine et al., 1984).

Table 4. The $Q_{10}$ relationships of photosynthesis and respiration in *Lissoclinum patella* colonies collected from exposed sites in Palau. The $Q_{10}$ values were obtained from the log transforms of rate *vs.* temperature plots over the ranges indicated. Colonies were incubated for 30 min at the experimental temperature prior to measurement. The coefficients of determination ($r^2$) for the regression lines of log rate *vs.* temperature are provided. All slopes were significantly different from zero ($p < 0.01$) except as indicated (ns).

| | $Q_{10}$ | Temperature range (°C) | $r^2$ |
|---|---|---|---|
| Photosynthesis | — | 15–45 | 0.46 (ns) |
| | 3.52 | 15–30 | 0.95 |
| | 1.62 | 30–45 | 0.91 |
| Respiration | 1.97 | 15–45 | 0.97 |

For high-light colonies the maximum value, obtained by using the $H_{comp}$ period (10.2 hr) for calculating daily gross photosynthesis, is 43.6%. For colonies from shaded habitats the minimum and maximum values are 36.8% ($H_{sat}$ = 7.4 hr) and 55.6% ($H_{comp}$ = 11.2 hr), respectively (see Table 3). Values of 63–69% have been calculated for some coral species by using daily photosynthetic periods of 12.7 hr (Muscatine et al., 1981), while a CPAR of 12–31% (on the basis of 20–50% translocation rate, and a photosynthetic period of 11.6 hr) was determined for another *Prochloron*/ascidian association, *Didemnum molle* (Olson and Porter, 1985).

*Temperature responses of L. patella*

Colonies of *L. patella* taken from water at ambient temperatures of 28–30°C and exposed to a range of temperatures between 15 and 45°C for 30–45 min showed no significant ($p > 0.01$) discontinuity in the log rate vs. temperature plots. The $Q_{10}$ was 1.97 ($r^2 = 0.97$ for the regression line) for colony respiration between 15 and 45°C. In a previous study, a $Q_{10}$ of 1.66 ($r^2 = 0.96$) was obtained for respiration of the symbiont (Alberte et al., 1986). Over this same temperature range, the regression ($r^2 = 0.46$) of log $P_{max}$ vs. temperture for *L. patella* colonies was not significant, indicating a discontinuity in the relationship. Regressions of colony $P_{max}$ between 15 and 30°C and between 30 and 45°C were highly significant. The $Q_{10}$ for the 15–30°C range was 3.52 ($r^2 = 0.95$), and that for the 30–45°C range was 1.62 ($r^2 = 0.97$). The $Q_{10}$ for photosynthesis of the symbiont isolated from this host also showed a discontinuity at 30° C, while respiration did not (Alberte et al., 1986). Collectively, these data show that the symbiont's

photosynthesis is nearly twice as sensitive to subambient temperatures as colony respiration.

## 4. Discussion

The diversities of *Prochloron*/ascidian symbioses and of natural light environments in the Kamori Channel, Palau, West Caroline Islands, offer an ideal field situation for comparisons of photosynthetic performance of different species in different light regimes. The two light environments examined here were the same as those we studied previously, so we could compare P-I responses of colonies with known data for the isolated symbiont (Alberte et al., 1986).

The fully exposed sites were never shaded and received maximum photon flux levels of 2300 $\mu$E $m^{-2}s^{-1}$, yielding total daily quantum fluxes (PAR) of 73.0 E $m^{-2}d^{-1}$. The shaded sites received a maximum of 450 $\mu$E $m^{-2}s^{-1}$ and total daily quantum fluxes approaching 10.5 E $m^{-2}d^{-1}$. Comparisons of light adaptation data for *L. patella* colonies revealed that neither P-I nor carbon balance (P:R ratios and CPAR) reflected the 7-fold differences in daily quantum flux. Instead, the responses showed only 30–40% differences, more similar to those of the *in hospite* daily photosynthetic quantum fluxes (total daily flux available between $H_{comp}$ and $H_{sat}$). The P-I and light adaptation responses of the symbiotic algae must, therefore, be interpreted not in terms of incident irradiation levels or total quantum fluxes, but in terms of *in hospite* light levels and daily quantum fluxes, which do not exceed the maxima utilizable for photosynthesis (i.e., flux levels less than or equal to $I_k$). The same conclusions were drawn from data for light regime, P-I, photosynthetic unit (PSU), and carbon balance in *Prochloron* cells isolated from *L. patella* colonies from high- and low-light environments (Alberte et al., 1986). Therefore, the feature of light regime which is most important in determining light adaptation characteristics is daily photosynthetic quantum flux and not instantaneous flux levels or total quantum fluxes (cf., Dennison and Alberte, 1985; Alberte et al., 1986).

The photoadaptive responses in all of the species studies were consistent, though not identical, with that observed for the symbionts freshly isolated from high- and low-light colonies of *L. patella* (Alberte et al., 1986). Evidently, *Prochloron* from any of these hosts is phenotypically plastic in response to its light environment, and is capable of positive photoadaptation. All colonies from high-light regimes showed shallower initial slopes of the P-I curves and higher $I_c$ and $I_k$ values than colonies from lower light regimes,

demonstrating that the algae of low-light colonies are more efficient in utilizing low photon fluxes. Thus, the P-I features of the *Prochloron*/ascidian associations can be predicted from the isolated symbiont's responses to light environment when the symbiont P-I relationships are determined on freshly isolated cells obtained from symbioses maintained under natural light regimes (Fisher et al., 1985; Alberte et al., 1986). Cells isolated from hosts and maintained in laboratory culture often show P-I relationships which differ from those obtained from freshly isolated symbionts that have adapted to *in hospite* light environments (e.g. Chang et al., 1983; Muller-Parker, 1985; Fisher et al., 1985).

It is not clear whether the observed interspecific differences in photoadaptive responses, particularly the sensitivities to high fluence rates, can be interpreted as an indication of genotypic differentiation among the symbionts. In colonies of *D. similis* and *L. punctatum* collected from low-light environments, the sensitivity of *Prochloron* to photoinhibition is precisely what would be expected from observations made on the isolated symbiont. Isolated *Prochloron* cells from low-light *L. patella* colonies, when measured in dilute suspension ($10^3$ cells $ml^{-1}$), show photoinhibition at about 500–600 $\mu E\ m^{-2}s^{-1}$ (Alberte et al., 1986), while *in hospite* in *D. similis* and *L. punctatum* they show significant photoinhibition at 700–1200 $\mu E\ m^{-2}s^{-1}$. Colonies of both of these ascidian specie are thin (1–2 mm thick), flat and transparent, and probably attenuate only a small portion of the incident radiation. The differences observed in the light threshold for photoinhibition are, therefore, probably related to the degree of light attenuation by the host and possible self-shading of *Prochloron* cells within the colony, rather than being indicative of the presence of high-light sensitive genotypes. Our data for six different *Prochloron*/ascidian symbioses provide little evidence for distinct photosynthetic ecotypes of the symbiont.

Among the species examined, differences between chlorophyll-based photosynthetic rates of whole symbioses and those of isolated symbionts indicate that packaging of *Prochloron* cells within the hosts can strongly influence their photosynthetic capacity. As mentioned above, light attenuation by the host (60–80% in *L. patella*, Alberte et al., 1986) and potential self-shading by the symbiont can reduce photosynthetic capacity *in hospite*, and here we show that in none of the associations are $P_{max}$ values as great as those of the isolated symbiont. The $P_{max}$ values of isolated *Prochloron* cells were obtained on cell suspension with densities of $10^{3-4}$ cells $ml^{-1}$ (Alberte et al., 1986), while cell densities *in hospite* were at least 100-fold greater. Muller-Parker

(1984, 1985) found similar results when comparing P-I relationships of the symbiotic sea anemone *Aiptasia pulchella* and the freshly isolated zooxanthellae, *Symbiodinium microadriaticum*. Photosynthesis in the sea anemone symbiosis did not show photoinhibition at flux levels where the isolated symbiont was significantly photoinhibited. Fisher et al. (1985) observed that the zoothanthellae (*S. microadriaticum*) freshly isolated from the giant clam, *Tridacna gigas*, showed lower $P_{max}$ values but steeper initial slopes of the P-I curves and lower $I_k$ values than that obtained from the intact symbioses. Surprisingly no photoinhibition of photosynthesis was observed in the isolated symbionts even at 3000 $\mu E\ m^{-2}s^{-1}$. Fisher et al. (1985) estimated that up to 80% of the light incident upon the clams, depending on size, was attenuated by the host tissue, significantly reducing the light levels for the symbionts *in hospite*. Thus, these examples demonstrate that the symbionts show light adaptation features characteristic of the light environment *in hospite* rather than that incident on the hosts.

Other features of the symbiont's environment may also strongly influence its photoadaptation and photosynthetic performance. For example, *Prochloron* cells *in hospite* are not as well stirred to reduce boundary layer and diffusion limitations as they can be *in vitro*, despite their extracellular location in the cloacal cavities. The possible role of host pumping behavior influencing carbon and nutrient exchange by the symbionts requires examination. Certainly, increases in oxygen tension around cells of the symbiont, or decreases in the availability of inorganic carbon (despite $CO_2$ produced by host respiration), will have profound effects on photosynthetic activity. It is also possible that substances derived from host metabolic activity can regulate photosynthesis, either through direct effects on photosynthetic electron transport, or through effects on carbon fixation and subsequent metabolism (e.g., by the removal of glycolate produced by the symbiont; Fisher and Trench, 1980). A recognition of such regulatory effects of the host on autotrophy of the algae is essential to our understanding of the nature and productivity of these symbioses.

In a previous study it was shown that P-I response of *Prochloron* from *L. patella* were mediated through changes in PSU organization (Alberte et al., 1986). *Prochloron* from low-light colonies had more light-harvesting capacity (larger PSU sizes) while cells from high-light colonies contained more photosystem I and II reaction centers. These macromolecular changes in PSU organization are probably responsible for most of the observed P-I relationships, though in general PSU features cannot be predicted on the basis of P-I

relationships alone (Gallagher and Alberte, 1985). In view of the known PSU features of *Prochloron*, we can attribute increased chlorophyll-based photosynthetic performance in *L. patella* colonies from high-light environments to increased numbers of reaction centers in the cells, while the increase in $I_k$ may result from the PS I and PS II unit sizes being smaller than those from low-light colonies. The high $P_{max}$ and $I_k$ values obtained for the other high-light species (*D. virens* and *T. cyclops* in particular) may be attributable to similar changes in PSU organization. However, the high $P_{max}$ of *D. similis* colonies (from low-light regimes) may be more strongly influenced by *in hospite* effects on oxygen exchange, as the photosynthetic capacity of these colonies approaches most closely that of isolated high-light *Prochloron* cells (Alberte et al., 1986).

Colony P:R ratios based on daily net photosynthesis and colony respiration are all above 1.0, suggesting that sufficient carbon is fixed by the symbionts to support most of the carbon demands of the hosts. For alga/ascidian symbioses studied here and elsewhere (e.g., Thinh and Griffiths, 1977), this interpretation is based on comparable studies of unicellular autotrophs, but it does not present a reliable physiological picture of these symbiotic associations because it fails to take into account the amount of carbon translocated from the symbiont to the host and consumed by the host's respiration (Muscatine et al., 1981). However, if carbon release rates are known for the symbiont species and if they fall into a similar range for a variety of host species, then these P:R ratios may be useful in comparison with P:R ratios and CPAR values obtained for closely related species where host respiration and carbon translocation were considered.

For high- and low-light *L. patella* colonies the P:R ratios obtained, taking into account host respiration, ranged from 1.48 to 2.78 (see Table 3). Again, these P:R ratios indicate that more than enough photosynthate is produced to support the carbon requirement of the host. When CPAR values are determined by using a value of 20% as the amount of carbon translocated to the host from the symbiont (based on carbon release rates of isolated *Prochloron* from *L. patella*; Alberte et al., 1986), then 30 to 56% of the carbon requirement of host respiration can be met by *Prochloron* photosynthesis. Similar determinations on *Didemnum molle* yielded a P:R ratio of 0.62 and a CPAR value of 12% (Olson and Porter, 1985). In the latter case, the *Prochloron* is not necessarily an obligate symbiont of *Didemnum molle*, and other work has indicated that this didemnid can grow asymbiotically (Bachmann et al., 1985; Olson, 1987).

The CPAR values and P:R ratios obtained for *L. patella* are within the range reported for zooxanthellae/coral symbioses (Muscatine et al., 1981; Muscatine et al., 1984) and for the giant clam (*Tridacna gigas*) symbiosis (Fisher et al., 1985). Comparisons of the P:R ratios (based solely on total colony $P_{max}$ and respiration; Table 2) of the other species examined here with those of *L. patella* indicate that *Prochloron* may make an important and even obligatory contribution to the organic nutrition of these other hosts, with the possible exception of *L. voeltzkowi* ($P_{net}$:$R_{colony}$ = 0.04). Carbon release rates determined on isolated *Prochloron* cells, or carbon translocation rates determined *in situ*, are all in the range 7–50% (cf., Fisher and Trench, 1980; Kremer et al., 1983; Griffiths and Thinh, 1983). The colony respiration rates obtained here (see Table 1) indicate that all of the didemnid host species have similar respiratory demands, except for *L. voeltzkowi* which has a respiratory rate double the mean of the other species (10.7 $\mu$mol $O_2$ mg $chl^{-1}h^{-1}$). In the other five species, the animal respiratory demand for organic carbon and the percent carbon release by the symbionts are similar. Therefore, we estimate that between 20 and 60% of the carbon requirement of all the hosts is met by *Prochloron*.

*Prochloron*/ascidian symbioses are restricted to warm tropical sea coasts where water temperatures rarely drop below 21° C; (Kott, 1977, 1982; Lewin, 1979; Lewin et al., 1983; Ryland et al., 1984). Since photosynthesis of *Prochloron* isolated from *L. patella* is extremely sensitive to chilling, it was suggested that the symbiosis must be obligatory and that the symbiont's thermal tolerance controls the distribution of its hosts (Alberte et al., 1986). The $Q_{10}$ values for photosynthesis and respiration of colonies of *L. patella* support this proposition. The present data show that colony photosynthesis is nearly twice as sensitive ($Q_{10}$ = 3.52) to lower than ambient temperatures (< 30°C) as is colony respiration ($Q_{10}$ = 1.97). At ambient or higher temperatures the $Q_{10}$ of colony photosynthesis ($Q_{10}$ = 1.62) is similar to that of colony respiration ($Q_{10}$ = 1.97), and nearly identical to that of the isolated symbiont (Alberte et al., 1986). Collectively, these findings demonstrate that the low temperature sensitivity of symbiont photosynthesis can restrict the thermal distribution of *L. patella*.

McCourt et al. (1984) demonstrated that in the northern Gulf of California the seasonal occurrence and abundance of epizoic populations of *Prochloron* corresponded to periods of warm water and were unrelated to host surface area or abundance. Since there is little evidence for the existence of different photosynthetic ecotypes of *Prochloron* in different ascidian hosts, it is likely

that the distribution of other *Prochloron*/ascidian associations is similarly limited to warm tropical waters, and indicates further that the endosymbiotic associations are probably obligatory as the host have not been found in colder waters.

Though the data presented here do not directly assess the contributions of *Prochloron*/ascidian symbioses to community productivity, they indicate a high degree of photosynthetic potential and autotrophy for these associations. If the minimum net carbon production per day (based on a daily photosynthesis period of 7 hr) is calculated for *L. patella* colonies and expressed on host area basis, a production rate of 8.3 $\mu$g C $cm^{-2}d^{-1}$ is obtained. Colonies with daily photosynthetic periods approaching 11 hr, more typical of the light regimes on the reef flats in Palau, could have production rates as great as 13.1 $\mu$g C $cm^{-2}d^{-1}$. These compare favorably with a value of 31.6 $\mu$g C $cm^{-2}d^{-1}$ for the hermatypic coral, *Stylophora pistillata* (Falkowski et al., 1984). Therefore, *Prochloron*/ascidian symbioses can make significant contributions to benthic productivity in areas where they are found. In addition, the findings indicate that *Prochloron*/ascidian associations can meet the definition of true mutualistic symbioses. In one case, *L. patella*, at least 30% and perhaps as much as 56% of the host respiratory carbon demand can be met by *Prochloron*, and by comparisons of colony P:R ratios we may conclude that, in all but one of the symbioses examined, the symbionts make comparably important contributions to the nutrition of their hosts.

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