

***Prochloron*-Ascidian Symbioses: Photosynthetic Potential and Productivity**

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Abstract—We determined the chlorophyll content of didemnid ascidians with symbiotic algae (*Prochloron* sp.) from tropical marine waters around Palau, Western Caroline Islands. Several species contain as much chlorophyll per unit dry weight as many herbaceous crop plants and more than other symbiotic associations such as lichens, green hydra, etc. Their chlorophyll *a/b* ratios (3–9) were generally much higher than those of angiosperms (2–4). Where they abound, *Prochloron*-ascidian symbioses could make a major contribution to the productivity, especially in localized areas of tropical marine waters characterized by low nutrient levels and high irradiance.

Introduction

Until a few years ago, didemnid ascidians with “zoochlorellae” were barely mentioned in lists of symbiotic associations of invertebrates with algae (e.g., Droop, 1963; Taylor, 1973; Trench, 1979). More recently, interest in these protochordates has grown, largely because of the discovery that their algal symbionts (*Prochloron* sp.) are prokaryotes capable of synthesizing chlorophyll *b* as well as chlorophyll *a*, a feature hitherto thought to be confined to eukaryotic chlorophytes such as green algae and higher plants (see review by Lewin, 1981). However, even among marine biologists familiar with many of the diverse symbiotic associations found in tropical coastal waters and reefs, few are yet aware of the potential ecological importance of symbiotic didemnids in such regions (Lewin, 1979; Kott, 1980).

In fringing reef areas and shallow lagoons surrounding the islands of Palau, Western Caroline Islands, several species of symbiotic didemnids are abundant. Along some of the lagoon shores, many rhizophores and lower branches of the mangrove *Sonneratia* bear a mosaic coating of a soft green didemnid, *Diplosoma virens* (Tokioka, 1942). On nearshore coastal flats, leaves of the seagrasses *Halophila* and *Enhalus* can be found partly enveloped by grey-green colonies of *Lissoclinum voeltzkowi*, while in nearby areas these seagrasses can be equally heavily laden with spherical colonies of yet another symbiotic species, *Didemnum molle*. Along parts of the Kamori Channel, at depths of about 1 m, dead coral surfaces are blanketed by

another green didemnid, *Trididemnum cyclops*. This same species also carpets large patches of the bottom of at least one of the marine lakes (Hamner, 1982).

In the localized areas where they are found, symbiotic didemnids could contribute significantly to primary productivity, since they are abundant and contain large numbers of photosynthetic cells (Lewin, 1981) with high rates of photosynthesis (Thin et al., 1981, Griffiths and Thin, 1983). There is evidence that these algal symbionts, which line cloacal cavities and other spaces of their host colonies (Kott, 1980; Lewin, 1981), release fixed carbon (Thin and Griffiths, 1977; Fisher and Trench, 1980; Kremer et al., 1982; Griffiths and Thin, 1983) assimilable by their hosts at levels between 7% (Pardy and Lewin, 1981) and 51% (Griffiths and Thin, 1983). These values are comparable to those, sometimes exceeding 20–30%, determined for hermatypic corals (Taylor, 1973; Muscatine, 1980). In coral reef systems, the net productivity ($\text{g C m}^{-2} \text{d}^{-1}$) may be 5 to 50 times as high as in the overlying waters (Marsh, 1976; Lewis, 1977; Trench, 1979), and may reach levels comparable to those reported for agricultural systems, kelp beds and seagrass communities (Dawes, 1981). We present here evidence, based on the chlorophyll contents in eight species of symbiotic didemnid ascidians, indicating an important role of *Prochloron*-ascidian associations in the primary productivity of certain tropical marine benthic communities.

Materials and Methods

Didemnid ascidians were collected in February, 1982, from a small coastal reef flat in the Kamori Channel, Palau, Western Caroline Islands. Colonies immersed in seawater were transported a few km from the field to running seawater tanks at the laboratory of the Micronesian Mariculture Demonstration Center, Koror. *Prochloron* sp. cells were removed from the various host species by squeezing the colonies. The ratios of fresh (wet) to ash-free dry weight were determined on the same samples. Ash weights were determined after calcining at 330°C for 1 hr. Chlorophyll contents and chlorophyll *a/b* ratios were determined in extracts in 90% (v/v) acetone by using equations of Jeffrey and Humphrey (1975). Some comparative data were obtained from collections made in Singapore in 1977, or from the published literature.

Results and Discussion

Among the 8 species of didemnids examined, the dry weight/wet weight ratios (Table 1) are roughly equivalent to those of most invertebrates and many angiosperms (Evans, 1972). Most of the values are less than 17%, the lowest values being those of *Didemnum molle* (8%) and *Diplosoma similis* (>3%) which are respectively full of mucilaginous material and a less viscous algal suspension. The one high value (42%), that of *Lissoclinum bistratum*, is attributable to the high content of calcareous material (84% of the dry weight) in the colonies of this species.

Table 1. Didemnid ascidians with symbiotic prochlorophytes: some physical and chemical data. (Unless otherwise specified, the data were based on specimens collected at Palau, W. C. I.)

Host ascidian: Genus and species	<i>Prochloron</i> cell diam (μm) ¹	Dry wt	Organic	Chl. <i>a</i>	Chl. <i>b</i>	Chl. <i>a</i>
		Wet wt %	Dry wt % ²	Organic mg/g ³	Organic mg/g ³	Chl. <i>b</i>
<i>Didemnum molle</i> (white)	16-28	15	73	5.0	1.3	3.9
<i>Didemnum molle</i> (brown)	16-22	8	42	3.6	1.15	3.1 ⁴
<i>Diplosoma similis</i>	9-18	>3	51-72	5.3	1.3	4.1 ⁵
<i>Diplosoma virens</i>	9-14	17	74	1.5	0.3	5.0 ⁶
<i>Lissoclinum bistratum</i> ⁷	14-20	42	16	1.2	0.15	8.8
<i>Lissoclinum patella</i>	13-22	10	63	0.8	0.25	3.3
<i>Lissoclinum punctatum</i>	18-30	14	63	3.75	0.75	5.0
<i>Lissoclinum voeltzkowi</i>	9-13	14	65	2.6	0.6	6.4
<i>Trididemnum cyclops</i>	9-18	16	54	1.8	0.4	4.8 ⁸

¹ Cell diameters are presented in lieu of specific/subspecific names; the taxonomy of *Prochloron* has still to be worked out.

² Most of the inorganic material is calcium carbonate (aragonitic spiculospheres).

³ Determined spectrophotometrically (Jeffrey and Humphrey, 1975).

⁴ Cf. 2.6-2.7 (Thorne et al., 1977)

⁵ Determined chromatographically by Dr. H. Paerl.

⁶ Cf. 6.7-12.0 (Thorne et al., 1977)

⁷ Collected in Singapore, 1977.

⁸ Cf. 5.5-7.0 (Thorne et al., 1977)

The chlorophyll *a/b* ratios (Table 1), 3.1 to 8.8, are in the same range as those reported for various *Prochloron* preparations examined earlier (summarized by Lewin, 1981), being higher than those typical of terrestrial plants (2.2 to 4.0 in Kirk and Tilney-Bassett, 1978) and of marine and freshwater green algae (1.5 to 2.8 in Wood, 1979; Alberte, unpublished). The amounts of chlorophyll *a* (per gm ash-free dry weight or per unit biomass) of the symbiotic associations range from 0.8 to 5.3 mg/gm (Table 1). These values are comparable to those found in the symbiotic *Paramecium bursaria*+zoochlorella complex (5 mg/gm; D. Weis, Cleveland, Ohio, pers. commun., July 1982) and in some whole angiosperms (comprising nonphotosynthetic as well as photosynthetic tissues) such as duckweed (*Lemna minima*, 5.5 mg/gm, our data) and barley (*Hordeum vulgare*) leaves (5.0-7.0 mg/gm, our data) and they are higher than that of the highly productive seagrass *Zostera marina* (Mazzella et al., 1981; Dennison and Alberte, 1982). The average value for the chlorophyll *a* content, 2.8 mg/gm, is higher than those reported for lichens (e.g., 0.4-1.2 mg/gm in *Peltigera canina*, Wilhelmson, 1959), the symbiotic green sponge *Spongilla lacustris* (0.55-1.2 mg/gm in Frost and Williamson, 1980), and the dinoflagellate-containing sea-anemone *Anthopleura elegantissima* (~1.0 mg/gm, estimated from data of Fitt et al., 1982). The relatively high levels of chlorophyll in symbiotic didemnids indicate a considerable potential for photosynthetic activity, which could make a significant contribution to their nutrition and thereby add to the

overall primary productivity of certain tropical benthic communities.

Though high chlorophyll content is not always correlated with high rates of photosynthesis (Kirk and Tilney-Bassett, 1978; Richardson et al., 1983), in many plants it is highly correlated with photosynthetic performance (Patterson, 1980). Light-saturated rates of photosynthesis in *Diplosoma virens* have been reported to be considerably greater than the dark respiration rates (P : R = 8, Tokioka, 1942, Tinh et al., 1981); in the other species listed in Table 1 (except for *Didemnum molle* and *Lissoclinum bistratum*, which were not examined) the P : R ratios are 10 or greater (Alberte, Cheng and Lewin, in preparation). Since in Palau these ascidians experience daily periods of light-saturated photosynthesis of about 12 hr (Alberte et al., in preparation), their daily (24 hr) P : R ratios are at least 5. Similar daily P : R ratios are found in such terrestrial angiosperms as wheat (*Triticum*) and maize (*Zea*) (C. Gudin, pers. commun.; de Witt et al., 1970) and in the seagrass *Zostera marina* (Dennison, 1984). In most of these symbiotic didemnids, net photosynthesis per unit chlorophyll *a* is comparable to, or exceeds, that of many marine macrophytic algae (Kirk and Tilney-Bassett, 1978; Dawes, 1981). On the same basis, net photosynthetic rates of the symbiont alone are comparable to those of typical cyanobacteria (e.g., *Anacystis nidulans*, Vierling and Alberte, 1980; marine *Synechococcus* spp., Alberte et al., 1984; Alberte et al., in preparation).

In Palauan reef-shelf waters, the majority of the symbiotic didemnid species, especially *Lissoclinum patella*, *Diplosoma virens* and *Didemnum molle*, are found at depths of less than 2–3 m where there is little attenuation of light intensity by the water column. (For example, at solar noon, under a cloudless sky, the light intensity [400–700 nm] at 2 m is 98% of that at 2 cm; Alberte, unpublished data). In isolated *Prochloron* cells and in *Lissoclinum patella* and *Diplosoma virens* colonies, photosynthesis saturates at light intensities of 500 to 1000 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Alberte et al., in preparation). These symbiotic associations are evidently adapted to high-light environments, maximizing their photosynthesis, and hence their primary production, under the light conditions prevailing in shallow reef waters.

Another feature of some of these symbiotic ascidians which may help them to maintain high rates of photosynthesis is their motility with positive phototaxis. Colonies of *Diplosoma virens* can move along substrate surfaces at rates up to a few mm per day (Birkeland et al., 1981; Tinh et al., 1981). This could help to keep them in well illuminated situations and reduce or even eliminate potential shading by other organisms or from burial under sediment.

Relatively high growth rates around 2.3% per diem have been reported for *Diplosoma virens* (Tinh et al., 1981); similar rates have been documented for such photoautotrophs as macrophytic algae (deBoer et al., 1978). Some of the nutrients supporting the growth of these animals presumably derive from photosynthetic activity of their algal symbionts. However, the presence of fecal pellets in their guts indicates *D. virens* ingests microplankton (e.g., bacteria and algae) providing an additional source of nutrition for the maintenance and growth of the host. Future work is needed to clarify the nutritional economy of symbiotic didemnids.

Although the presence of symbiotic algae in didemnid ascidians has been recognized for some time, their uniqueness has been only recently appreciated. The nutritional role of these prochlorophyte symbionts in the growth of their hosts remains unclear, but there is now considerable evidence indicating that they can contribute significantly to the primary production of some tropical littoral communities. In the present report we demonstrate that at least eight species of *Prochloron*-didemnid symbioses have photosynthetic potentials (based on their chlorophyll content) equalling or exceeding primary productions of other marine or terrestrial species.

ACKNOWLEDGMENTS

We are grateful to Dr. P. Kott for help with the identification of the didemnid hosts, to Dr. James Marsh Jr. for helpful discussions, and to the Government of Palau for the use of facilities at the Micronesian Mariculture Demonstration Center, Koror. Financial support from NASA (HNAGW-181) to RAL, and from NSF (OCE 8214914) to RSA, is gratefully acknowledged.

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