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Ascidian–Algal Symbioses: II. Photoadaptation in *Didemnid ascidians* with Red Cyanophytes

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Abstract

Three species of ascidians, *Didemnum* aff *sphaericum*, *Didemnum viride* and *Tridemnum tegulum*, with symbiotic red, unicellular cyanophytes exhibit "multi-photoadaptive" responses which allow them to occupy a wide range of habitats. The symbiotic algae have the ability to alter the concentration of their accessory photosynthetic pigments, the phycoerythrins; together with the ability to alter the phycoerythrobilin/phycourobilin chromophore contents, the chlorophyll *a* concentration is not affected. The ascidian host, *T. tegulum*, increases spicule density with increasing light intensity.

Keywords: Ascidians, photoadaptation, *Tridemnum tegulum*, *Didemnum* aff *sphaericum*, *Didemnum viride*, photosynthetic pigments

1. Introduction

A number of colonial didemnid ascidians have been reported in association with red cyanophytes in both obligate and non-obligate symbioses (see Lafargue and Duclaux, 1979; Kott et al., 1984; Parry, 1984). Olson (1980, 1986) reported a number of responses to shading of the ascidian *Tridemnum solidum* which has the unicellular red cyanophyte *Synechocystis tridemni* as its symbiont: (1) distribution of algae within the colony, (2) ratio of organic to inorganic matter increases with decreasing light intensity, (3) Phycoerythrin concentration increases with decreasing light intensity; (4) colony morphology, (5) UV absorbing compounds are in higher concentration in light colonies.

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Symbesma et al. (1981), also working on *T. solidum*, quantified the pigment concentration of the symbiont for colonies taken from 3 different depth ranges. They found that the number of cyanophytes and the chlorophyll *a* per g wet weight of ascidian colony did not change significantly with depth. They did not investigate the phycobiliproteins in the *S. tridid*emni.

A large number of algal species, and in particular cyanobacteria, have the facility for chromatic adaptation; varying both the level of photosynthetic pigments and their ratios to one another under different light regimes. It is well established that shading causes an increase in the concentration of light harvesting proteins, such as, the phyobiliproteins (Larkum and Barrett, 1983).

I investigated the mechanisms of photoadaptation in 3 species of ascidians with red, unicellular, cyanophytic symbionts — *Didemnum* aff *sphaericum* (Tokioka, 1967), *Didemnum viride* (Herdman, 1906) and *Trididemnum tegulum* (Kott, 1984).

2. Materials and Methods

Collection of ascidians

The *Didemnum* aff *sphaericum* colonies were attached to the underside of boulders just below the low-water mark. The colonies were collected from the centre of boulders (completely shaded) and other colonies on the outer edge (only partially shaded).

The *D. viride* colonies were collected from the reef slope just below the low-water mark in full sun down to depths of 20 m where large sheeting colonies extend from full sun irradiance to under ledges where irradiance is extremely low.

The *T. tegulum* were found attached to coral rubble and boulders just below the low-water mark with a range of light intensities from full shade to almost full sun.

All 3 species were collected at Heron Island, Great Barrier Reef.

PAR (Photosynthetically Active Radiation)

PAR measurements were recorded before the ascidians were collected, without disturbing the habitat and thereby recording the natural light environment. All measurements were taken between 1000 and 1400 hr and no cloud cover. The light meter and sensor was specially constructed to allow for instantaneous measurements of PAR in a wide range of habitats e.g. under ledges, in small caves, under boulders.

The sensor was a BPW 21 photometric diode, measuring light in the range 400–700 nm. The spectral response of the sensor was measured with a Varian Cary 17 spectrophotometer. The meter and sensor were calibrated against a LiCor 1000 data logger equipped with the LiCor LI 192 SA underwater quantum sensor.

Isolation of pigments

In all 3 species the symbiotic algae are embedded in the test, together with calcium carbonate spicules. The algae are not able to be removed from the ascidian hosts; therefore, either whole colonies (*T. tegulum*) or sections of colonies (*D. viride* and *Didemnum* aff *sphaericum*) were extracted within 3 hr of collection.

The phycobiliproteins were exhaustively extracted with cold 0.1 M phosphate buffer (pH 7.0) by crushing in a glass homogenizer followed by freezing and thawing. The aqueous extract was obtained by centrifugation at 16000 g for 20 min at 4°C. The supernatant was made 30–50% saturated with ammonium sulfate. The red/maroon precipitate was collected by centrifugation at 4°C and suspended in 1 mM phosphate buffer – 0.1 M NaCl (pH 7.0) and dialysed exhaustively against the same buffer at 4°C.

The visible spectra were recorded on a HP 8450A diode array spectrophotometer. The phycobiliprotein concentrations (phycoerythrin and phycocyanin) were determined using the equations of Beer and Eshel (1985):

$$PE = [(A_{564} - A_{592}) - (A_{455} - A_{592})0.20]0.12$$
$$PC = [(A_{618} - A_{645}) - (A_{592} - A_{645})0.51]0.15$$

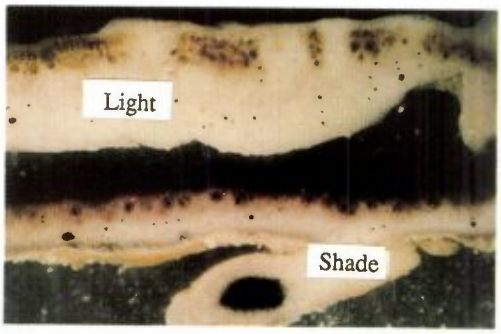
The residues from the aqueous extractions were immediately exhaustively extracted with cold acetone and absorption spectra were recorded immediately on a Varian DMS 90 spectrophotometer. The chlorophyll *a* concentration was calculated using the extinction coefficient of Jeffrey and Humphrey (1975): 90% acetone at 664.3 nm $\epsilon = 87.67\,\mathrm{lg^{-1}cm^{-1}}$. Total carotenoids were calculated using the equations of Parsons and Strickland (1963).

3. Results

Figure 1 shows the effects of light intensity on the spicule density and pigmentation in all 3 species. In *T. tegulum* the spicule density in the surface increases with increasing light intensity (Fig. 1(a)). The *D. viride* colonies are almost white, due to the $CaCO_3$ spicules, in full sun to dark purple in

Light Shade

(a) *Tridemnum tegulum* colonies showing increase in spicule density with increasing light intensity.



(b) *Didemnum viride* section through colonies showing increase in phycoerythrin concentration with decreasing light intensity.

Light Shade

(d) *Tridemnum tegulum* colony showing loss of phycobiliproteins in light.

Light Shade

(c) *Didemnum* aff *sphaericum* colonies showing increase in phycoerythrin concentration with decreasing light intensity.

Table 1(a). *T. tegulum*, *Didemnum* aff *sphaericum* and *D. viride* concentration of photosynthetic pigments of the symbionts from different light environments. Values are means ± standard deviations ($n = 6$).

Host species	PAR[a]	Chlorophyll *a*[b]	Total Carotenoid[b]	Carotenoid/ Chlorophyll *a*
T. tegulum	light	348.3 ± 33.0	91.4 ± 14.0	0.26
	shade	365.2 ± 64.2	95.8 ± 17.9	0.26
D. aff *sphaericum*	light	133.7 ± 13.0	37.4 ± 7.1	0.28
	shade	143.0 ± 26.0	36.4 ± 6.0	0.26
D. viride	light	280.7 ± 27.4	79.8 ± 9.1	0.28
	partial shade	271.8 ± 30.8	82.1 ± 8.4	0.30
	shade	283.7 ± 34.6	80.6 ± 5.5	0.28

a. Light: 2,100-600 μE $m^{-2}s^{-1}$; Partial shade: 600-200 μE $m^{-2}s^{-1}$; shade <200 μE $m^{-2}s^{-1}$
b. Concentration in μg g^{-1}wet weight ascidian tissue.

full shade (Fig. 1(b)). The *Didemnum* aff *sphaericum* colonies show color changes from dark purple in full shade to yellow when in higher irradiance (Fig. 1(c)).

Photosynthetic pigments

The amount of chlorophyll *a* (μg g^{-1} for all 3 species ranged from 111–395 μg g^{-1} (Table 1(a)). There was no significant variation in chlorophyll *a* or total carotenoid with light intensity (one way Anova, 0.05) within each species (Table 1(a)).

The phycoerythrin concentrations showed dramatic variation, in all 3 species, with changing light intensity/quality (Table 1(b)). The differences within each species are highly significant (one way Anova, 0.05). The most dramatic variation occurring in *D. viride*, ranging from 42–358 μg g^{-1} wet weight.

The phycocyanin concentrations are relatively low in all 3 species (Table 1(b)), with no significant variation within each of the 3 species (one way Anova, 0.05).

PAR measurements

A range of measurements are presented in Table 2. These PAR values reflect the wide range of light intensities to which these 3 species are exposed.

Table 1(b). *T. tegulum*, *Didemnum* aff *sphaericum* and *D. viride* concentration of photosynthetic pigments of the symbionts from different light environments. Values are means ± standard deviations ($n = 6$).

Host species	PAR[a]	CU-PE[b]	PC[b]	PC/CU-PE
T. tegulum	light	733.7 ± 95.7	184.5 ± 16.3	0.25
	shade	1175.5 ± 124.7	188.0 ± 23.0	0.16
D. aff *sphaericum*	light	110.7 ± 16.0	31.0 ± 7.0	0.28
	shade	259.7 ± 38.0	33.8 ± 6.8	0.13
D. viride	light	77.8 ± 16.5	12.7 ± 6.3	0.16
	partial shade	174.2 ± 12.8	12.3 ± 6.5	0.07
	shade	279.7 ± 44.0	12.2 ± 5.2	0.04

a. Light: 2,100–600 μE $m^{-2}s^{-1}$; Partial shade: 600–200 μE $m^{-2}s^{-1}$; shade: <200 μE $m^{-2}s^{-1}$.

b. Concentration in $\mu g\ g^{-1}$ wet weight ascidian tissue.

Table 2. PAR values for a range of habitats, Heron Island, Great Barrier Reef

Habitat/Depth	PAR[a]
Full sun plus sky	2300
Subsurface[b]	1800–2100[c]
Shade on reef crest	40–200
12 m top coral head	550–750
12 m under ledge	135–150
18 m top coral head	350–450

a. μE $m^{-2}s^{-1}$ b. approx. 10 cm c. fluctuations due to wave action

4. Discussion

Photoadaptation has been studied in the symbionts *Symbiodinium* spp., in corals, clams and sea anemones and there is no strategy which is common to all species (Wethey and Porter, 1976; Titlyanov et al., 1980; Falkowski and Dubinsky, 1981; Dustan, 1982; Chang et al., 1983). In the 3 species of ascidians in this study a number of adaptations can be identified, both qualitatively and quantitatively. These species occur in shallow and deep water and the light environment of these ascidians varies widely even at the

same depths. Some species occupying fully shaded habitats while others receive constant high levels of illumination (Table 2). The light environment of the ascidian host is not the same light environment experienced by the cyanophytic symbiont. The light received by the symbiont is further modified by the ascidian tissue and self-shading due to the packing of the algal cells.

This investigation was conducted *in situ* using the intact associations. The quality and quantity of light could not be successfully manipulated; with different colored filters, due to the habitats occupied by these species. Further, the ascidians could not be maintained in aquarium systems and the algae have, as yet, not been cultured; this precluded any laboratory investigation of these ascidian-algal associations. It was therefore necessary to isolate the photosynthetic pigments of the symbionts from freshly collected material.

There are a number of responses to changing light conditions in these ascidian species which are additional to the photoadaptation by the algae. The most obvious changes are in spicule density and color of the colonies. In *T. tegulum* the spicule density increases with light intensity (Fig. 1(a)). The spicules may be acting as a sun screen for the algae and/or a sun screen for the ascidian; protecting the ascidian tissue from the destructive effects of the UV light. An extreme exception was observed with a colony of *T. tegulum* growing from full shade into light (Fig. 1(d)). One half of the colony in the shade was dark maroon while the half in the sun was green; the algae reduced its phycoerythrin content dramatically as spicule density had not increased sufficiently to protect the algae. The different colors of *D. viride* colonies are due to the distribution of the algal cells in the ascidian (Fig. 1(b)) and the concentration of the phycobiliproteins. One extensive, sheeting colony of *D. viride* at 12 m covered an area of approx. 3 m^2; the color of the colony changed from white, where it was on the top of a coral head receiving full irradiance (550–750 $\mu E\ m^{-2}s^{-1}$), to red/maroon under ledges where light intensity was very low (150 $\mu E\ m^{-2}s^{-1}$). While it appears that the algal cell density is reduced in *D. viride* colonies exposed to high light environments, with colonies appearing white, the chlorophyll *a* concentrations indicate this is not the case (Table 1(a)). The phycoerythrin (CU-PE) concentration is markedly reduced and therefore the algal cells are not as obvious in the spicule packed test of the colony (Table 1(b)).

In all three species the chlorophyll *a* and total carotenoid concentrations were not affected by light quality/quantity (Table 1(a)). There is no significant difference in the ratio of total carotenoids/chlorophyll *a* in light and shade colonies and even between the 3 species (Table 1(a)). Olson (1986)

reported a total carotenoid/chlorophyll *a* ratio of 0.27 for light colonies of *Tridemnum solidum* and a significantly different ratio of 0.21 for shade colonies.

However, the phycoerythrin concentrations, in all 3 species, increased dramatically with decreasing irradiance (Table 1(b)). The concentration of CU-PE in *T. tegulum* is markedly higher than both *Didemnum* aff *sphaericum* and *D. viride*. This is due to the fact that the latter 2 species are packed with $CaCO_3$ spicules to a point where some colonies are almost brittle; thus having the affect of giving an extremely high wet weight of "tissue". Therefore, one can not make any direct comparisons between species based on these figures. From these results it can be seen that the symbionts have the ability to modulate the synthesis of phycoerythrin but not of phycocyanin. This is in contrast to the results reported by Olson (1986) for *S. trididemni* in *T. solidum*, where he found increases in both phycoerythrin and phycocyanin concentrations with decreasing light intensity. The phycocyanin concentrations for the symbiont from all 3 species in this study are extremely low relative to the phycoerythrin concentrations, with phycocyanin/phycoerythrin ratios ranging from 0.04 to 0.28 (Table 1(b)). Olson (1986) reports phycocyanin/phycoerythrin ratios of 1.02 (in light) and 0.67 (in shade). Tandeau de Marsac (1977) identified 3 groups of cyanobacteria with respect to chromatic adaptation:

I do not adapt
II adapt by changing PE synthesis
III modify both PE and PC.

Therefore, these symbionts can be classified as Group II with respect to chromatic adaptation.

Another mechanism of photoadaptation in cyanobacteria is the ability to alter the proportion of the different chromophores within a single phycobiliprotein molecule (Yu, et al., 1981). This mechanism has been observed in thee ascidian symbionts, with the ratio of phycoerythrobilin (PEB)/phycourobilin (PUB) varying with light intensity (Parry, 1988; Cox, et al., 1985).

It is the alteration in the concentration of the phycoerythrin, together with the ability to alter the PEB/PUB, that permit maximal utilization of the available light energy for photosynthesis. It is this "multi-photoadaptive" response in these ascidian-algal symbioses that allows these ascidians to successfully occupy such a wide range of habitats.

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