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# The Ecology of the Tropical Compound Ascidian *Tridemnum solidum*. III. Symbiotic Association with Unicellular Algae

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ABSTRACT: We investigated the relation between *Tridemnum solidum* (Van Name) – a tropical compound ascidian common on the reefs of Curaçao – and its endosymbiotic unicellular algae. Colonies collected from 3 depths (< 10 m, 15–17 m, > 20 m) were simultaneously analyzed for number of algae and amount of chlorophyll *a* $g^{-1}$ wet weight. Both number of algae (mean 2.78 × $10^6$ cells $cm^{-2}$) and amount of chlorophyll *a* (mean 150 µg chl. *a* $g^{-1}$ wet weight) did not change significantly with depth. *T. solidum* tadpole larvae contained significantly higher quantities of chlorophyll *a*. Additional pigment analysis of samples from shallow and deep water (< 10 m, > 20 m) revealed the presence of carotenes and zeaxanthin in quantities that remained the same regardless of depth, but the ratio of zeaxanthin to chlorophyll *a* changed significantly from 0.14 (shallow) to 0.10 (deep). Two-dimentional TLC confirmed the absence of chlorophyll *b* and *c* in these algae. The photosynthetic $O_2$ production of colonies from the 3 depth ranges was measured between July and September 1978, mainly in the laboratory. We used closed systems under artificial light conditions varying from 0–240 µE $m^{-2}s^{-1}$ (400–700 nm). Positive production rates were measured from 40–240 µE $m^{-2}s^{-1}$ (our highest experimental light level). Additional field experiments showed ascidian colonies from the shallowest sample (< 10 m) to consume oxygen at light intensities of 600 µE $m^{-2}s^{-1}$ and more. Maximum measured mean photosynthetic $O_2$ production was ± 0.09 mg $O_2$ $cm^{-2}h^{-1}$ at 240 µE $m^{-2}s^{-1}$ for colonies of 15–17 m and > 20 m. This $O_2$ production was significantly higher than the mean maximum production of colonies from 10 m (0.04 mg $O_2$ $cm^{-2}h^{-1}$).

## INTRODUCTION

Symbiosis between algae and invertebrates is a very common phenomenon (Buchner, 1953; Droop, 1963; McLaughlin and Zahl, 1966; Taylor, 1973; Trench, 1979). In the marine environment such relations, especially between algae and coelenterates, are particularly abundant in tropical shallow water. The association between 'zooxanthellae' and Scleractinia is a striking example of the importance of the algae for the invertebrate host in calcification and energy budget (e.g. Muscatine, 1973; Buddemeier and Kinzie, 1976; Muscatine and Porter, 1977).

Algal cells are known to occur in the test of ascidians from the Indo-Pacific (Michaelsen, 1920; Smith, 1935). Eldridge (1966), reviewing the didemnid ascidians of this region, also localized algae in the cloacal systems. He suggested the algae to be species specific because algae are transmitted by the larvae. Kott (1977), examining didemnids from the Great Barrier Reef (Australia), quotes Newcomb and Pugh (1975) who recorded the algae living in cloacal systems and tests to be blue-green algae. Kott (1977) considers the association to be species specific. Such algal-ascidian symbiosis was unknown from the Atlantic Ocean until recently when Lafarque and Duclaux (1979) and Sybesma and Bak (1979) described associations between unicellular algae and didemnid ascidians from, respectively, Guadeloupe and Curaçao.

There are few quantitative data available on the significance of the relations of algae and non-coelenterate marine hosts, and virtually none on algae-ascidian relations (Millar, 1971; Withers et al., 1978). We investigated the number of algae and quantities of photosynthetic pigments present in colonies of *Tridemnum solidum*, as well as the photosynthetic $O_2$ production of the algal-ascidian unit. We also report on other aspects of the biology of this compound ascidian (Bak et al., 1981; Van Duyl et al., 1981).



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## MATERIALS AND METHODS

### Location and Quantity of Algae

We prepared histological sections to locate the position of the algae in the colonies. Samples were preserved in Bouin's solution. To facilitate cutting of the test, which is densely packed with spicules, we treated the material with R. D. O. (decalcifier, Bethlehem Ltd). After exposure to a series of ethanol and xylol baths, sections (10 µm) were stained with haematoxyline/eosine and embedded in DePex for inspection with the stereo-microscope.

To count the numbers of algal cells we made a homogenate blending 20 ml ascidian tissue with 80 ml seawater. Large spicules were separated from the homogenate (0.1 mm mesh filter). Algal cells were counted in aliquots of this homogenate with a haemocytometer (Fuchs-Rosenthal, Hofheim/Taunus). To convert number of algae to colony surface area, a conversion factor was calculated.

All samples used were from colonies collected by SCUBA diving at the southwest coast of Curaçao (Carmabi Reef Buoy, 0 and 4). The colonies were grouped for 3 depth ranges: shallow reef (< 10 m), medium depth (15–17 m) and deeper reef (> 20 m). In sampling the colonies we avoided the colony margins. All samples were processed within 4 h after collection.

### Pigments

Initially we determined only the quantities of chlorophyll *a* in *Trididemnum solidum* colonies from the 3 depth ranges (< 10, 15–17, > 20 m). Samples of known wet weight were extracted in 90 % acetone and the amount of chlorophyll *a* was measured spectrophotometrically following standard methods (Vollenweider, 1967). These procedures were repeated with known numbers and wet weights of *Trididemnum solidum* larvae. The larvae were gathered using a larvae collector (Van Duyl et al., 1981) at a depth of 18 m, except for 2 samples which consisted of larvae from various depths.

In addition, samples from the shallowest and deepest part of the range of *Trididemnum solidum* (< 10 m, > 20 m) were tested for the presence of other chlorophylls using two-dimensional Thin Layer Chromatography (Jeffrey, 1968, 1974) These samples were also analysed with High Performance Liquid Chromatography (Abaychi and Riley, 1979) to determine the quantities of chlorophylls, chlorophyll degradation products and carotenoids per unit ascidian surface area.

### Oxygen Production

We used colonies from the 3 depth ranges which were carefully detached from their natural substrata. Specimens were transported submerged in seawater to the laboratory and kept in running seawater, in the dark, for 1–2 h before starting an experiment. To measure oxygen consumption and production the colonies were subsequently placed in an incubator (described in Vooren, in press) to be exposed to a given light-intensity for a given time (incubation time). Our light source in the laboratory was a series of flood lights (Philips PAR 38); experimental light intensities varied from 0 to a maximum of 240 µE $m^{-2}s^{-1}$ at 400–700 nm, the range of photosynthetically active radiation (LI-185 quantum-meter, LI-COR Ltd). Each colony was used in a series of 1 h incubations at different light intensities. Because higher light intensities than 240 µE $m^{-2}s^{-1}$ are reached at depths < 10 m we exposed colonies of our shallow water sample, using the incubator, to such intensities on the reef at a depth of 4 m. In all experiments temperatures were at the same level as open seawater (27°–29 °C).

Water movement inside the closed incubator was maintained by a magnetic stirrer. The change in the amount of dissolved oxygen was measured as a parameter of consumption or production of organic carbon. Dissolved oxygen was determined by Winkler titration (Lind, 1974).

## RESULTS

### Algae and Algal Pigments

Examination of microscopic slides of *Trididemnum solidum* showed unicellular algae to be abundant in the test tissue (Fig. 1). These algae are spherical, 7–10 µm in diameter and greenish coloured in fresh condition. The number of algal cells per unit colony surface ranged from $1.0 \times 10^6$ to $4.4 \times 10^6$ per $cm^2$ (Table 1). We found no significant change in quantities of algae between the 3 depths (one way Anova, $p > 0.75$).

The amount of chlorophyll *a* $g^{-1}$ wet weight ranged from 45.5–302.4 µg (Table 1) There was no significant variation with depth (one way Anova, $p > 0.75$). Because algal counts and chlorophyll determinations were performed with samples of the same colony, we could calculate the mean quantity of chlorophyll *a* per algal cell in a colony Wet weight was converted to surface area and it appeared that the chlorophyll *a* content of the algal cells ranged from $5.7 \times 10^{-6}$ to $31.2 \times 10^{-6}$ (Table 1).

Analysis of the amount of chlorophyll *a* $g^{-1}$ wet

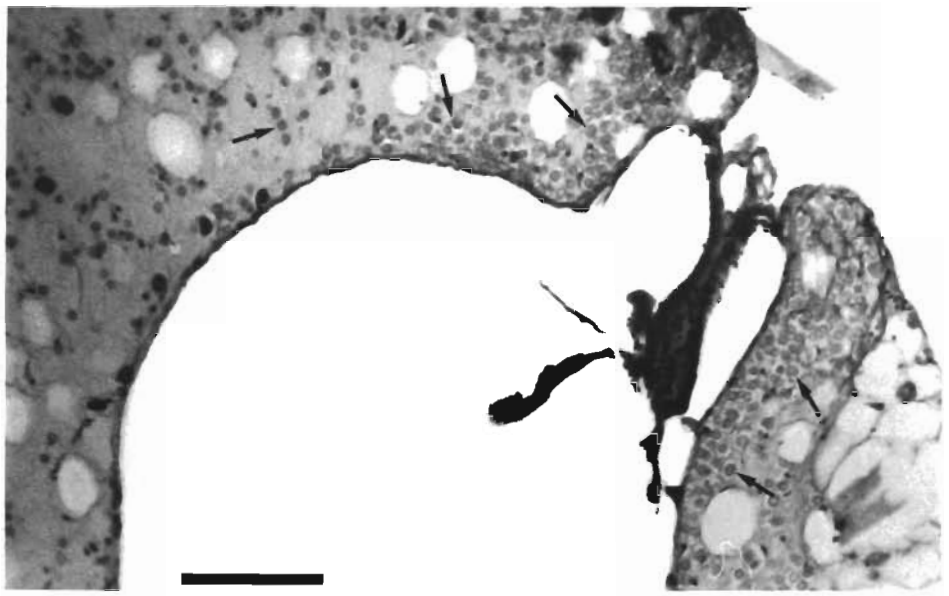

Fig. 1 *Tridemnum solidum*. Section showing empty cavity with remnant of zooid and surrounding test. Colony surface at upper right. Arrows indicate algal cells in test tissue. Bar = 100 µm

Table 1. Mean densities of algae and quantities of photopigments (± SD) in *Tridemnum solidum* samples from various depths (n in parentheses). There is no significant difference between the various depths (one way Anova)

| Parameter | Depth (m) | | |
|---|---|---|---|
| | <10 | 15–17 | >20 |
| Density of algae (cells $cm^{-2} \times 10^6$) | 2.7 ± 1.6 (6) | 2.7 ± 1.2 (6) | 2.9 ± 0.9 (6) |
| Chlorophyll *a* (µg $g^{-1}$ wet weight) | 133.2 ±86.6 (6) | 165.9 ±85.7 (6) | 151.1 ±60.3 (6) |
| Chlorophyll *a* (µg $cell^{-1} \times 10^{-6}$) | 16.1 ± 8.8 (6) | 18.3 ± 8.8 (6) | 15.7 ± 8.6 (6) |
| Chlorophyll *a* (µg $cm^{-2}$) | 24.1 ± 6.8 (4) | – | 27.9 ± 4.1 (4) |
| Phaeophytin *a* (µg $cm^{-2}$) | 21.4 ± 4.5 (4) | – | 20.4 ± 4.8 (4) |
| Carotene (µg $cm^{-2}$) | 5.4 ± 1.4 (4) | – | 5.8 ± 1.3 (4) |
| Zeaxanthin (µg $cm^{-2}$) | 3.3 ± 0.8 (4) | – | 2.8 ± 0.6 (4) |

weight in *Tridemnum solidum* larvae showed this to be much higher than in *T. solidum* colonies (Table 2). The mean quantity of chlorophyll *a* $g^{-1}$ wet weight of larvae was 607.06 µg $g^{-1}$ wet weight (range 330.92–952.64) The comparable, significantly lower values for the colonies with a mean 150.06 $g^{-1}$ wet weight are not even overlapping

Neither TLC nor HPLC analysis revealed the presence of other chlorophylls. Such absence of chlorophyll *b* and *c* is characteristic for blue-green algae. That the symbionts belong to the Cyanophyceae is confirmed by E. M. photographs (courtesy Dr. A. Svoboda) which showed the algae to be prokaryotic. The quantities of chlorophyll *a* degradation product and carotenoids present in our samples are shown in Table 1. There is no significant difference in pigment quantities with depth. However, the ratio of zeaxanthin to chlorophyll *a* was significantly higher (student's

Table 2. *Tridemnum solidum*. Chlorophyll *a* $g^{-1}$ wet weight of larvae and chlorophyll *a* per larva (mean values). The mean number of algal cells per larva in a sample was calculated assuming the chlorophyll *a* content per cell to be similar in larvae and larger colonies. All samples were collected at 18 m, except those marked with asterisks which are from various depths

| | Chlorophyll *a* (µg $g^{-1}$ wet weight) | Chlorophyll *a* µg larva $^{-1}$ | Algal cells larva$^{-1}$ |
|---|---|---|---|
| | 932.64 * | 0.324 * | 19401 * |
| | 662.22 * | 0.243 * | 14551 * |
| | 859.55 | 0.253 | 15150 |
| | 330.92 | 0.149 | 8922 |
| | 459.54 | 0.315 | 18862 |
| | 530.78 | 0.303 | 18144 |
| | 473.76 | 0.317 | 18982 |
| Total mean | 607.06 | 0.272 | 16287 |

*t* test $p < 0.01$) in the shallow samples (0.14) than in the deep samples (0.10).

### Oxygen Production

The relation between $O_2$ production and light intensities for *Tridemnum solidum* colonies of the 3 depths is shown in Fig. 2. A oneway Anova established that there is no difference in production levels betwen the colonies from deep and medium deep water ($p > 0.25$). However, there was a significant difference ($p < 0.001$) between the production at these depths (> 15 m) and that in the shallow reef (< 10 m).

Colonies from both deep and medium deep water appeared to reach saturation at 240 µE $m^{-2}s^{-1}$. Colonies from the shallow reef, where much higher light intensities are reached, showed photoinhibition at light levels beyond 200 µ $m^{-2}s^{-1}$. This was confirmed by data obtained with the shallow water sample at higher light intensities (600 and 1000 µE $m^{-2}s^{-1}$). At these light levels the algal-ascidian associations had a net oxygen consumption.

The ambient light levels for the different depths are shown in Fig. 3 (van Buurt, unpubl.). It appears that the ascidian colonies from the 2 lower depths (15–17 m, > 20 m), which reached their compensation point at a very low light level (± 10 µE $m^{-1}s^{-1}$) and showed no photoinhibition at a light intensity of 240 µE $m^{-2}s^{-1}$, will produce oxygen for at least a considerable part of the day. In the deep reef (25 m) where light intensities never exceed the saturation level for the colonies from deeper water, oxygen production will be above compensation level for 11 h from 6.30 to 17.30 h. In the

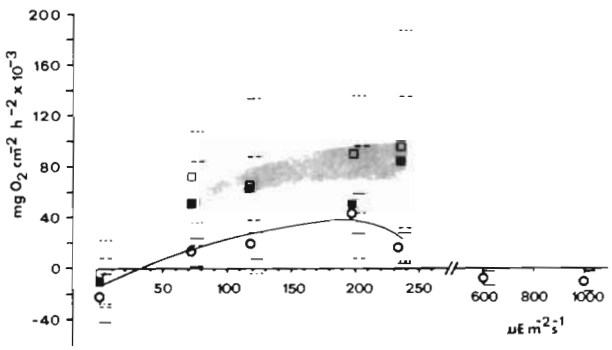


Fig. 2. *Tridemnum solidum*. Light/$O_2$ production for colonies from 3 depths: < 10 m (circles), 15–17 m (open squares), > 20 m (closed squares). Sample size n = 6 for each light intensity, except 600 and 1000 µE $m^{-2}s^{-1}$ (n = 2). Oxygen production values for colonies of deeper water (15–17 m, > 20 m) are significantly higher than for shallow water colonies (one way Anova, $p < 0.001$). There is no significant difference between the medium (15–17 m) and deep water sample (> 20 m). Horizontal dashes: standard deviation (— < 10 m, --- 15–17 m, --- > 20 m)

shallow part of the reef (< 10 m) where light intensities in excess of 600 µE $m^{-2}s^{-1}$ occur, *Tridemnum solidum* colonies will show net oxygen consumption during the middle of the day. For example, a colony at a depth of 5 m, assuming that the algal-ascidian associa-

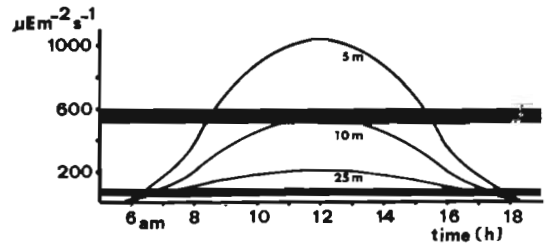


Fig. 3. Mean radiation intensities (400–700 nm) at 5, 10, and 25 m depth during the day (after van Buurt, unpubl.). Between the light levels indicated by horizontal bars oxygen production exceeds respiration

tion starts net oxygen consumption again at 500 µE $m^{-2}s^{-1}$ (Figs. 2, 3), will produce oxygen from 6.00 to 8.30 h, and again from 15.30 to 17.40 h. This means that the total time of net oxygen production is only half as long at this depth as it is in colonies at e.g. 25 m depth.

## DISCUSSION

The extensive literature on symbiosis of algae with marine invertebrates appears to emphasize problems of translocation and conservation of nutrients, aspects of cell biology, etc. Data on densities of symbiotic algae, quantities of chlorophyll and the variation of these parameters in different habitats (depths) are scarce. Nevertheless, there is high consistency in the available values, varying with few exceptions from 1–8 × $10^6$ cells $cm^{-2}$ (Drew, 1972; Dustan, 1975; Stiévenart, 1975). These values stem from alcyonarian and scleractinian corals, but algal densities in *Tridemnum solidum*, 1.02–4.43 × $10^6$ cells $cm^{-2}$, and clionid sponges (Elema, unpubl.) are of the same magnitude. Drew explained the small variation between his samples (0.90–2.40 × $10^6$ cells $cm^{-2}$) suggesting 2 layers of algal cells as the upper limit tolerated by the coral association. The rather larger variation in the data of Dustan, 1.93–11.80 × $10^6$ cells $cm^{-2}$, all data of one species, *Montastrea annularis* (Ellis and Solander), shows at least this symbiotic association to be much more adjustable to algal densities. In *T. solidum* the algal densities cannot be influenced, as may be the case in scleractinian corals, by variation in the calyx and polypary structure. It is conceivable that the comparatively simple, essentially

two-dimensional structureless surface of the ascidian colonies is the reason the values on algal density are in the lower part of the range of published data. The overall similarity in number of algal symbionts per unit surface area in marine invertebrates suggests an underlying principle which is yet to be explained.

Variation of densities of symbionts with depth (light intensity) has been studied in a few scleractinian corals with confusing results. In some species algal numbers are reported not to vary with depth (Drew, 1972; Redalje, 1976), in others to decrease with depth (Stiévenart, 1975), for other species there appears to be a maximum algal density at intermediate depths (20 m; Bak et al., unpubl.) and there may be genetic differences involved between shallow and deep water populations of the coral-algal association (Dustan, 1975).

Chlorophyll quantities in scleractinian corals may or may not increase with depth. This means that sometimes there is a consistent quantity of chlorophyll present per algal cell (Bak et al., unpubl.) while other studies showed a variation (Redalje, 1976); sometimes there are differences between the deep and the shallow association. This phenomenon may be explained by the existence of differences between various forms of the algal symbiont, *Gymnodinium microadriaticum* Freudenthal (e.g. Schoenberg and Trench, 1980). It also remains to be examined to what degree scatter in the published data is related to microhabitat variation.

The algae in *Tridemnum solidum* appear to be unrelated to the coral symbionts, and it must be investigated whether similar physiological races – or possibly species – appear among the various symbionts found in didemnid ascidians. An important difference between the blue-green – ascidian and the *Gymnodinium* – scleractinian relation is the much higher concentration of chlorophyll *a* per surface area in the former (Stiévenart, 1975; this paper: Table 1). The number of algal cells per surface area is similar in both associations and the difference is caused by the much higher chlorophyll content per cell of the *T. solidum* symbionts. That this difference may be significant is supported by the results of Thinh and Griffith (1977). They found a high chlorophyll *a* content per algal cell, $7 \times 10^{-6}$ µg, well comparable with our range of 5.7–31.2 $\times 10^{-6}$ µg cell$^{-1}$. These are much higher chlorophyll *a* contents than reported for coral symbionts (e.g. $1.5 \times 10^{-6}$ µg cell$^{-1}$; Stiévenart, 1975). Data on the chlorophyll *a* content of larvae of symbiotic associations are unknown to us. Our own data show the concentration of chlorophyll to be much higher in the larvae than in the colonies. This suggests firstly that the ascidian is highly dependant on its algal symbionts, and secondly that infection of aposymbiotic larvae is unlikely. This means that also the algae must have a close relation with their animal host, at least on the habitat level.

In spite of the similarity in algal densities and chlorophyll *a* concentration of *Tridemnum solidum* colonies over the reef, we found a significant difference in oxygen production between the shallow and deep reef colonies. Oxygen consumption in the dark was of the same magnitude in both groups. There are several possible explanations: Firstly, the significant change in zeaxanthin to chlorophyll *a* ratios with depth may be induced by environmental conditions such as light levels (Parson et al., 1977; Wettern and Weber, 1979); Hager and Meyer-Bertenrath (1967) mentioned zeaxanthin quantities to be positively related with light intensity; a possible function of zeaxanthin is shielding off the photopigments, and the colonies from deeper water, lacking such a filter, may have been more sensitive to our artificial light source. Secondly, the photosynthetic quotient becomes higher when non-carbohydrates are produced (Parson et al., 1977) and blue light favours protein synthesis over carbohydrate synthesis (Wallen and Green, 1971 a,b,c); the deep water colonies are possibly adapted to relatively blue light and the high oxygen production may be the result of a difference in the photosynthetic quotient. Unfortunately, it was logistically not feasible to test these alternatives and some reservation must be made in our interpretation.

Oxygen production in the ascidian symbiotic association is relatively high (maximum values of 0.04–0.09 mg $O_2$ cm$^{-2}$h$^{-1}$) compared with similar data on production per surface area in various algae (0.015–0.043 mg $O_2$ cm$^{-2}$h$^{-1}$; e.g. Marsh, 1968; King and Schramm, 1976; Wanders, 1976).

The significant difference in oxygen production of *Tridemnum solidum* with depths may indicate that a large part of the energy budget is easily provided by the algal symbionts in the deeper colonies. Oxygen production as such, however, does not give any information on the efficiency or magnitude of energy translocation between alga and host. In Scleractinia it has been estimated that generally 35–40 % of the algal photosynthetate is contributed to the host (Wethey and Porter, 1976 a; McCloskey et al., 1978). Wethey and Porter (1976 b) suggest that the efficiency of the translocation may vary with depth. Compound Didemnidae are filter feeders (Millar, 1971) and it is not known whether material is exchanged within the algal-ascidian association.

P/R ratios give less information on the internal energy budget of symbiotic associations than is generally inferred (McCloskey et al., 1978). These authors point out that P/R ratios > 1 are required to suffice the metabolic requirements of the invertebrate-algal unit. Of course, high P/R ratios make it more likely that the

total metabolic demand is filled, assuming that a given percentage of photosynthetically fixed energy is translocated.

Approximate values for the P/R ratio of shallow and deep reef colonies of *Tridemnum solidum* can be calculated using the daily length of the oxygen production period (see Results), darkness respiration values (assuming a constancy of consumption rate over the 24 h period, the error introduced is probably small; Mergner and Svoboda, 1977) and oxygen production levels at the different light intensities (Fig. 2). Such calculations lead to a very high P/R ratio for deep reef colonies: ± 7, and a relatively low P/R, 1.0 for shallow reef colonies. The maximum P/R of 7 seems exceedingly high in view of most P/R values encountered in hermatypic scleractinians (McCloskey et al., 1978; their Table 1). But such maximum values, 8–8.5, have been found in the only other study on photosynthesis in didemnid ascidians (Thinh and Griffith, 1977). These authors studied shallow water colonies which photosynthesized at saturation level at very high intensities (± 2000 $\mu E\ m^{-2}s^{-1}$). Our shallow reef samples showed photoinhibition and net oxygen consumption at much lower light levels (± 500 $\mu E\ m^{-2}s^{-1}$), while we infer highest P/R ratios to occur in colonies from relatively deep water.

We conclude that the symbiotic association of *Tridemnum solidum* and blue-green algae is a very efficient oxygen producer and although the exact functioning of the symbiotic association over the reef slope remains obscure, alga and host are highly interdependent. There appears to be a great potential of energy translocation in the association, the magnitude of which may depend strongly on the reef habitat.

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