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## Photoadaptation of zooxanthellae in the sponge *Cliona vastifica* from the Red Sea, as measured in situ

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**Abstract** In the Red Sea, the zooxanthellate sponge *Cliona vastifica* (Hancock) is mainly present at > 15 m depth or in shaded areas. To test whether its scarcity in unshaded areas of shallower waters is linked to the functional inefficiency of its photosymbionts at high irradiances, sponges were transferred from 30 m to a six times higher light regime at 12 m depth, and then returned to their original location. During this time, photosynthetic responses to irradiance were measured as rapid light curves (RLCs) in situ by pulse amplitude modulated (PAM) fluorometry using a portable underwater device, and samples were taken for microscopic determinations of zooxanthellar abundance. The zooxanthellae harboured by this sponge adapted to the higher irradiance at 12 m by increasing both their light saturation points and relative photosynthetic electron transport rates (ETRs). The ETRs at light saturation increased almost fourfold within 15–20 days of transfer to the shallower water, and decreased back to almost their original values after the sponges were returned to 30 m depth. This, as well as the fact that the photosynthetic light responses within an individual sponge were in accordance with the irradiance incident to specific surfaces, shows that these photosymbionts are highly adaptable to various irradiances. There was no significant change in the number of zooxanthellae per sponge area throughout these experiments, and the different photosynthetic responses were likely due to adaptations of the photosynthetic apparatus within each zooxanthella. In conclusion, it seems that parameters

other than the hypothesised inability of the photosymbionts to adapt adequately to high light conditions are the cause of *C. vastifica*'s rareness in unshaded shallow areas of the Red Sea.

### Introduction

Marine sponges show a wide repertoire of associations with photosynthetic organisms such as filamentous cyanobacteria, zooxanthellae and other unicellular algae (e.g. Sarà and Liaci 1964; Arillo et al. 1993; Gaino and Sarà 1994; Hinde et al. 1994). For some species, it has been shown that the photosymbionts can provide the host with organic photosynthates (Wilkinson 1979, 1983) and, as a consequence, these sponges may thrive in areas where successful competition for nutrients and space is important for their establishment and growth. Cheshire et al. (1997) demonstrated how the bathymetric distribution of two sponge species harbouring cyanobacteria is restricted to depths not >25–30 m because of their need of light. Thus, those sponges were apparently only able to survive at depths where they could obtain at least 80% of their respiratory carbon requirements photosynthetically. Maldonado and Young (1998) noted that some mixotrophic sponges (sponges that have a facultative association with the photosymbionts) are also restricted to shallower areas. They translocated specimens of two keratose species to various depths down to 300 m and, contrary to the conclusions of the previous work, suggested that the absence of these species at great depths was not correlated with the presence of cyanobacteria. They indicated, rather, that it was due to the inhibition of larval dispersal or settlement due to physical parameters such as the colder water at those depths.

Regarding zooxanthellate sponges, it has been demonstrated that the fitness of a boring sponge harbouring zooxanthellae is greater than that of azooxanthellate specimens (Hill 1996). Hill and Wilcox (1998) observed that the sponge *Anthosigmella varians*, following a

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bleaching event after being transferred from 20 to 1 m depth, acquired an algal strain different from the original one.

Recently, the non-invasive technique of pulse amplitude modulated (PAM) chlorophyll fluorometry has become available also for underwater measurements (e.g. Ilan and Beer 1999). This, for example, has enabled the evaluation of the photosynthetic performance of zooxanthellae in corals at ambient light in situ, without the need of enclosing them in respiration chambers and thus inadvertently influencing factors such as water flow and temperature (Beer et al. 1998; Ralph et al. 1999). The only study so far to use PAM fluorometry on sponges was that of Beer and Ilan (1998), who measured in situ differences in photosynthetic responses of the photosymbionts of *Cliona vastifica* and *Theonella swinhoei* growing under naturally low irradiances ( $< 50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). It was found that specimens that grew under very low light conditions featured lower light saturation points and lower maximal photosynthetic rates than individuals growing under higher irradiances. Since marine sponges in general are very sensitive to removal from the sea to laboratories for experiments, PAM fluorometry offered a non-invasive means for estimating the photosynthetic performance of sponge photosymbionts in situ. Accordingly, we used this method here to measure the extent to which the photosymbionts of *C. vastifica* are photoadaptable to higher irradiances.

Like other species of its genus, the boring sponge *C. vastifica* harbours zooxanthellae (Sarà and Liaci 1964; Rosell and Uriz 1992). These zooxanthellae are embedded intercellularly, and are more abundant near the sponge surface. In the Red Sea, the distribution of this sponge extends down to at least 40 m, and it is mostly abundant between 25 and 30 m (Peretzman-Shemer, unpublished data), while it becomes rare in shallower depths, where it occurs mostly in shaded areas (Beer and Ilan 1998). In the present work, we investigated whether the cause of this depth distribution pattern could be linked with a functional inefficiency at higher irradiances of the photosymbionts harboured by *C. vastifica*. In order to do so, we determined in situ quantum yields ( $Y$ ) and relative photosynthetic electron transport rates (ETRs) of zooxanthellae in individuals that were transferred from deep water to a shallower zone with irradiances higher than those where this sponge is commonly found.

## Materials and methods

Six individuals of the sponge *Cliona vastifica* (Hancock) were translocated in the sea by SCUBA diving. The specimens originally grew between 20 and 30 m depth in the coral reef of Eilat, Israel (northern Red Sea,  $31^{\circ}35'N$ ;  $34^{\circ}54'E$ ). Three control specimens were transferred within the same depth (20 m, maintaining their original light regime). The other three (designated sponge a, b and c) were collected at 30 m, in habitats featuring maximal midday irradiances of  $25\text{--}90 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (400–700 nm, photo-

synthetic active radiation, PAR). These three sponges were transferred for 3.5 weeks to 12 m depth, and then returned to 30 m. The experiments were carried out between August and October 1999, at an ambient water temperature of  $25\text{--}27^{\circ}\text{C}$ . During this time, all photosynthetic measurements were done at the same time of the day (around 12:30 hours).

We used the underwater pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Germany) for measuring chlorophyll fluorescence parameters of the zooxanthellae (cf. Beer and Ilan 1998). Thus, the electron transfer quantum yield of photosystem II ( $Y$ ) was calculated as  $Y = (F_m' - F) / F_m'$ , where  $F$  is the fluorescence under a given ambient irradiance and  $F_m'$  is the maximal fluorescence in such light-adapted photosymbionts as registered during the application of a 0.8 s period of photosynthesis saturating light (ca.  $6000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ).

The photosynthetic responses to irradiance were measured as rapid light curves (RLCs), using the Diving-PAM's internal halogen light source. These curves were generated by first briefly covering the sponge surface with a "coral clip" (manufacturer's terminology), after which  $Y$  was immediately ( $< 2$  s) determined in darkness; this was followed by eight more consecutive  $Y$  determinations following pre-set increasing light levels lasting 10 s each. Relative photosynthetic electron transport rates (ETRs) were calculated as  $Y \times$  incident PAR. The eight incident irradiances of the RLCs were measured just before a series of  $Y$  measurements by placing the Diving-PAM's quantum sensor in front of the "coral clip". Ambient irradiances were also measured with the light sensor of the Diving-PAM, which had been calibrated earlier in the laboratory against the quantum sensor of a Li-Cor (USA) LI-189 quantum meter. Irradiances experienced by the sponges at 30 m were measured at midday for each sponge on two consecutive days (the day before and the day of the translocation to 12 m). At 12 m, both RLCs and ambient irradiances were measured at midday of every day for the first 9 days, daily during the third week, and from the 25th to the 27th day. Forty days after returning the sponges to their original location at 30 m, RLCs were measured again for two consecutive days on each sponge. Average irradiances were calculated from the in situ irradiances measured throughout these experiments.

In the above experiments, the "coral clip" covers ca.  $5 \text{ cm}^2$  of the sponge surface for some 80 s during the RLC measurement, and this reduces the water flow around the tissue that is measured by the Diving-PAM's optic fibre (ca.  $0.5 \text{ cm}^2$ ). In order to test to what degree this interference could influence the photosynthetic performance of the zooxanthellae, a separate experiment was performed in which RLCs were measured either with the "coral clip" or with the so-called "leaf distance clip", which leaves the sponge tissue open to the surrounding water. It was found that the use of the "coral-clip" did not significantly affect  $Y$  and, consequently, ETR values; at the four highest irradiances of the RLCs, values were 86–125% of those obtained in the "leaf distance clip" (at the same RLC irradiances and at a very low ambient irradiance of ca.  $7 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Likewise, it was ensured that the repeated 0.8 s saturating light period preceding each  $Y$  measurement of the RLC did not considerably affect the  $Y$  values; sets of eight saturating light periods during 80 s under a low ambient irradiance of  $15 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (around sunset at 10 m depth) caused a maximal decrease of  $Y$  by 8%. In addition to the photosynthetic measurements, histological analyses were performed. At 30 m depth (before the sponges were transferred to 12 m), after they had been at 12 m for 3.5 weeks, and 41 days after they had been returned to their original site at 30 m, samples were taken from each sponge ( $10 \text{ mm}^2$  area  $\times$  5 mm thickness) in order to measure the zooxanthellar density. The samples were fixed for 24 h in 4% formalin buffered in seawater, rinsed and preserved in 70% ethanol. After fixation, the samples were decalcified with sodium citrate and formic acid, and desilicated with 4% hydrofluoric acid (Ilan 1995). Algal abundance was determined by counting the zooxanthellae in each sample under an epifluorescent light microscope. Each sample was counted in five different, equally sized areas. The results were tested by a mixed-model ANOVA (Sokal and Rohlf 1995).

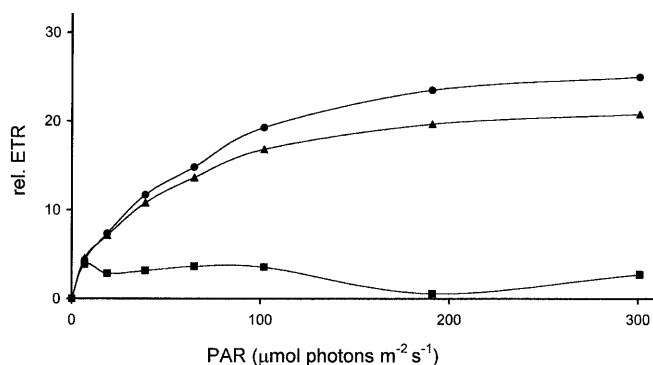
For three individuals of the sponge *C. vastifica*, RLCs were measured on two surfaces (facing upward, toward the incident sunlight, and down toward the bottom), so as to investigate whether various adaptations to light occur also within the same individual sponge.

## Results

The photosynthetic responses to light of the three individual *Cliona vastifica* sponges from 30 m depth are presented in Fig. 1. While there were differences in these responses, the saturation irradiances correlated with the ambient irradiances that each individual experienced in situ. Sponge c, which grew at a midday irradiance of ca.  $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , showed saturation at ca.  $180 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , while the corresponding values for sponges b and a were 45 and  $28 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  ambient light and 120 and  $< 20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  saturating light, respectively. Also the relative ETRs varied in accordance with the irradiance experienced in situ (Fig. 1).

The changing patterns of RLCs during the adaptation time of the sponges to 12 m depth, where the average midday irradiance was  $248 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , is exemplified for sponge c in Fig. 2. A similar pattern of increasing relative ETRs after transferring the sponges to 12 m depth was obtained also for specimens a and b, and the average maximal relative ETR values, i.e. the ETR recorded at the (high) irradiance that yielded the highest value, over the 3.5 weeks that the sponges were left at 12 m depth, are presented in Fig. 3. While these values increased gradually with time, and stabilised after 15–20 days (Fig. 3), the saturating irradiance increased more drastically within the first days of the transfer, and soon levelled out (Fig. 2).

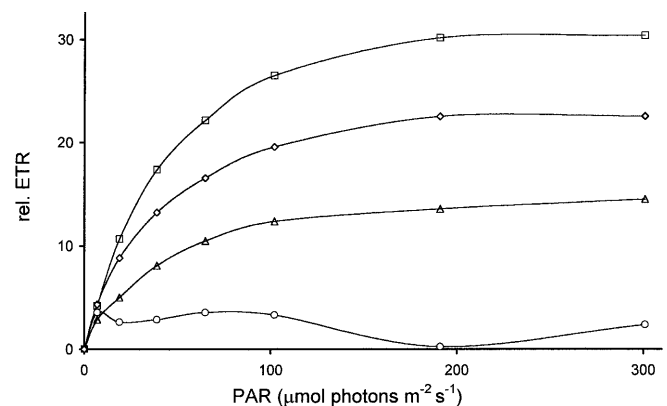
Following the 3.5 weeks at 12 m, the three sponges (a, b and c) were returned to their original habitat at 30 m depth. To test whether the adaptation of the



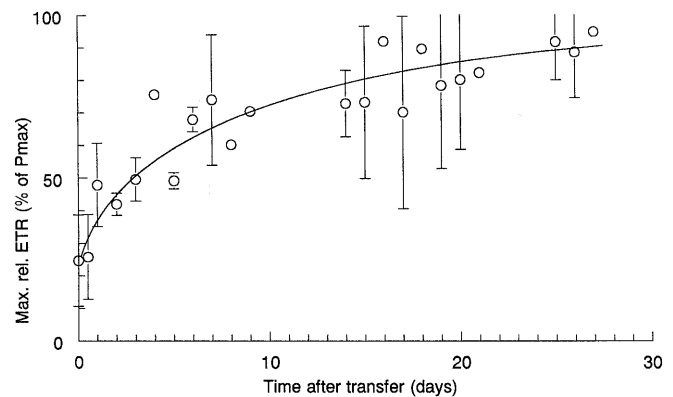
**Fig. 1** Relative photosynthetic electron transport rates (*rel. ETR*) as a function of irradiance (measured as photosynthetic active radiation, PAR) for *Cliona vastifica* sponges a (square), b (triangle) and c (circle), at a depth of 30 m, in surroundings characterised by noon irradiances of 28 (a), 45 (b) and  $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (c). The rapid light curves (RLCs) were generated by irradiating for 10 s at each irradiance

zooxanthellae to 12 m was reversible, relative ETRs were measured again 40 days after returning the sponges to the deeper location. At that time, maximal relative ETRs had decreased back to values close to those obtained prior to the transfer to shallow water (Fig. 4). In the control treatment, RLCs were measured for three *C. vastifica* individuals before and after relocation within the same depth level for a period of 3 weeks. Unlike the situation with the sponges transferred to shallower waters, the control sponges showed no marked change in the photosynthetic performance (results not shown).

Adaptations to various light environments occurred not only in different individuals exposed to different depths, but also within the same individual facing different light regimes. For example, RLCs obtained for three individuals (at 17–29 m depth, Fig. 5) showed



**Fig. 2** Relative photosynthetic electron transport rates (*rel. ETR*) as a function of irradiance (measured as photosynthetic active radiation, PAR) for *Cliona vastifica* sponge c measured before (circle), and 3 (triangle), 10 (diamond) and 25 (square) days after the transfer to 12 m depth. The rapid light curves (RLCs) were generated by irradiating for 10 s at each irradiance



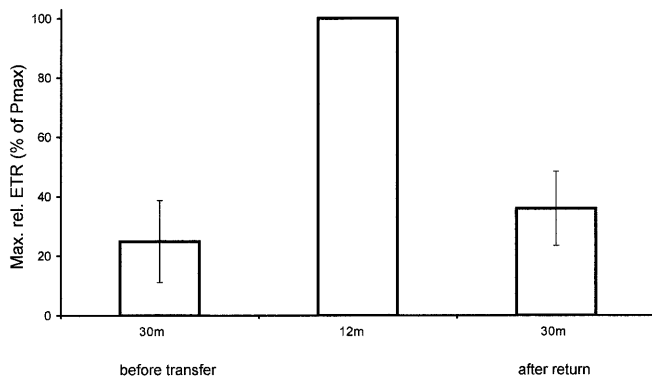
**Fig. 3** Maximal relative photosynthetic electron transport rates (*Max. rel. ETR*) of rapid light curves (RLCs), as derived at high irradiances, in percentage of maximal values reached during the adaptation time at 12 m depth ( $P_{\text{max}}$ ) for *Cliona vastifica*, as a function of days after transferring the sponges to 12 m depth (day 0 represents measurements taken at 30 m depth just before the transfer). Data points are averages of replicates; SD is given when data were available for all three individuals

higher relative ETRs in parts facing upward (receiving 45–140  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at the time measured) than in a part of the same individual facing down toward the bottom (receiving 1–14  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ).

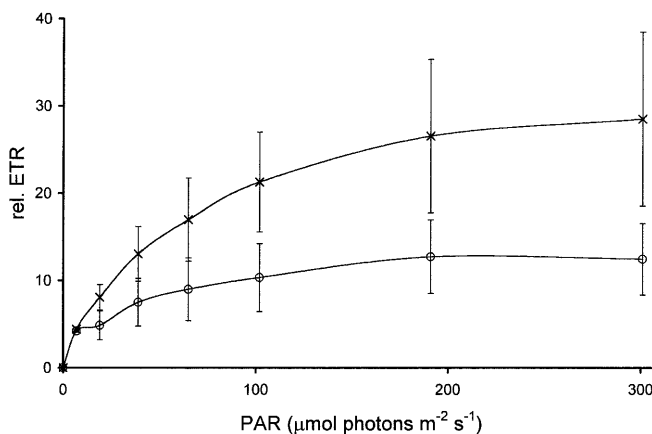
We found no significant difference in the abundance of zooxanthellae following translocations of the sponges; the number of zooxanthellae per area was the same before and after translocation of the sponges to the various depths (Fig. 6).

## Discussion

The zooxanthellae symbiotic with *Cliona vastifica* are highly adaptable to various irradiances. Firstly, this is apparent by the relationship between the photosynthetic

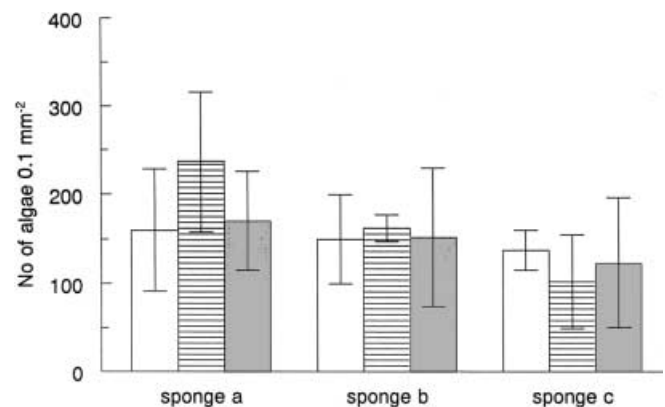


**Fig. 4** Percentage maximal relative ETRs of rapid light curves (RLCs), as derived at a high irradiance (*Max. rel. ETR*), for *Cliona vastifica* at 30 m depth at the beginning and end of the experiment relative to the highest values obtained at 12 m (100%). Values at 30 m are averages  $\pm$  SD of three individuals



**Fig. 5** Relative photosynthetic electron transport rates (*rel. ETR*) as a function of irradiance (measured as photosynthetic active radiation, PAR) for the upper (*cross*) and lower (*circle*) parts of *Cliona vastifica* sponges. Data are averages  $\pm$  SD of three different individuals from depths of 17–29 m. Natural irradiances measured for the upper parts of the sponges were between 45 and 140  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; for the parts of the sponge facing the bottom, irradiances were between 1 and 14  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

light responses of the zooxanthellae and the ambient light to which the various hosts were naturally exposed at 30 m depth. Secondly, these zooxanthellae seem to be able to gradually adapt to changes in the light field when translocated to shallower water. This is, for example, expressed in the increased ETRs at high irradiances of the RLCs and higher light-saturation points. At least the former response likely involves increased amounts of photosynthetic enzymes, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The time needed for such an adaptation to be completed was 15–20 days for sponges transferred to a habitat with sixfold the irradiance of their original site. Conversely, when the individual sponges were redeployed to their original location at 30 m, a decrease in saturating irradiances and maximal relative ETRs was observed. This further demonstrates the adaptability of the zooxanthellae to dimmer light as well. The responses of the present zooxanthellae differ from those of cyanobacteria found in other sponges, where it was shown that the maximal photosynthetic rate did not change among individuals along a depth gradient (Cheshire et al. 1997). Another adaptation strategy could involve a change in the relative abundance of two or more strains of zooxanthellae populating the sponges so as to favour those that can cope with higher irradiances. Such a strategy has been proposed for corals (Rowan and Knowlton 1995; Rowan 1998). As mentioned before, Hill and Wilcox (1998) described the event of reacquisition of a different algal strain following bleaching in a sponge. Therefore, another possibility would be that the original zooxanthellae present in the sponges at 30 m did not survive the increase in irradiance at 12 m depth, and were replaced by new recruited populations. This explanation seems, however, less likely since, directly after the transfer, we would then expect an initial, drastic decline in photosynthetic activity as a result of the death or expulsion of the original zooxanthella population. The



**Fig. 6** Number of zooxanthellae per 0.1  $\text{mm}^2$  surface area in three *Cliona vastifica* sponges (a–c) at 30 m depth before transfer (*open bars*), 3.5 weeks after transfer to 12 m (*horizontally striped bars*) and 40 days after the return to 30 m (*solid bars*). Data are averages ( $\pm$ SD) of five different, equally sized areas of each sponge (ANOVA,  $P > 0.05$ )

increase in photosynthetic rates at higher irradiances was not caused by an increase in zooxanthellar abundance either; we observed no significant change in the number of algae per given area before and after the translocations.

PAM fluorometry has been used before to measure photosynthetic responses of sponges to light (Beer and Ilan 1998), and the technique was also used here in order to follow the adaptation of the photosymbionts harboured in the sponge *C. vastifica*. Compared to conventional gas exchange ( $O_2$  and  $CO_2$ ) methods of measuring (net) photosynthetic rates, PAM fluorometry provides a quick, non-invasive means of estimating (gross) photosynthetic performance. One potential problem in using this method for photosymbiont-containing marine organisms is that many of them grow under low ambient irradiances (such as the present sponge) and, therefore, may be sensitive to the periods of saturating light needed in order to measure  $F_m'$ . Another potential disturbance is that the application of the "coral clip" restricts the water flow around the surface tissues during measurements of RLCs. However, it was found here that the series of eight saturating light periods, as well as the time period of coverage under the "coral clip", did not influence  $Y$  values significantly. Here, it was also found that a sponge specimen from a certain location showed different responses according to the orientation of the sponge surface. Therefore, when comparing the photosynthetic responses of individuals of different light environments, the occurrence of these "microclimates" of varying irradiance should be taken into account. In our adaptation study, we always measured the photosynthetic performance on the upward-facing part of the individual sponges and always did the measurements at the same time of the day. Also, we found that the RLCs better represented the adaptational process with time than did point measurements under ambient light; in the latter, even small differences in irradiance resulted in different ETRs, while in the RLCs, the incident PARs remain constant. By setting the time of each irradiance level to 10 s, there was enough time to perform up to ten RLCs per SCUBA dive at 30 m.

Using PAM fluorometry, we found that the limited presence of *C. vastifica* in shallow habitats in the Red Sea is not due to an inability of its photosymbionts to adapt adequately to the higher irradiances encountered there; the adult specimens transferred to such an area showed no obvious sign of stress even after 3.5 weeks. Rather, it seemed that at these depths the higher ETRs would increasingly contribute to the energy requirements of the hosts. It is therefore suggested that the distribution of this species primarily in deeper waters is linked to other features of juvenile sponges, such as the inability of larvae to settle in shallower environments, possibly due to higher competition for space in this mostly coral-dominated zone.

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