

## Photosymbiosis in Intertidal and Subtidal Tropical Sponges

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### Abstract

The frequency of marine sponges that harbour photosynthetic organisms was examined in a tropical area of the Western Indian Ocean. Out of 77 species from five different habitats in Zanzibar, 55 (71.5%) were found to have photosynthetic activity, as assayed by *in situ* pulse amplitude modulated (PAM) fluorometry (and validated by measurements of chlorophyll content). A significantly higher percentage of the intertidal (85%, n=27) than of the subtidal (64%, n=50) species was found to be photosynthetically active. In addition, *in situ* measurements of a common intertidal *Haliclona* sp. confirmed that photosynthetic activity was maintained during prolonged periods of air exposure at low tide. The fact that each habitat contained its own characteristic species (only eight species were found in more than one habitat) suggests that the generally high presence of photosymbionts in all the various intertidal communities may be an important component to the successful adaptation of those species to life in the intertidal. We propose two possible explanations for the higher presence of photosymbiotic species in the intertidal: 1) Intertidal sponges may be more dependent on autotrophic symbionts to meet their energetic needs because they are unable to filter-feed during air exposure at low tide; 2) The photosynthetic symbionts provide UV protection in an environment where UV radiation is high.

Keywords: Chlorophyll, marine sponges, PAM fluorometry, photosymbionts, symbiosis

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## 1. Introduction

In oligotrophic waters, such as those characteristic of coral reef habitats, a high number of invertebrates including corals (Falkowski et al., 1990), sea anemones (Furla et al., 1998), bivalves (Leggat et al., 2000) and sponges (Wilkinson, 1979, 1983) harbour photosymbiotic organisms. The occurrence of photosynthetic activity in those animals may be related to the relative scarcity of organic food particles such as phytoplankton and zooplankton. A comparison between the Great Barrier Reef (GBR) of Australia and the Caribbean showed that the latter area had a higher sponge biomass, but "phototrophic" sponges (those in which over 50% of their respiratory O<sub>2</sub> output could be balanced by photosynthetic O<sub>2</sub> production, according to Wilkinson 1987) were virtually absent.

On the other hand, the GBR was rich in photosymbiotic species. It was suggested that the higher sponge biomass of the Caribbean was due to a higher amount of nutrients in the water, and that the high percentage of phototrophic sponges in the GBR was related to the lower productivity in the water column of that area (Wilkinson, 1987). In such nutrient-poor environments, marine sponges would thus benefit from the supplemental contribution of photosymbionts as an additional source of energy. Similarly, it may be advantageous for photosynthetic organisms to utilise the metabolic waste products from sponges in such oligotrophic waters.

The intertidal may present an extreme habitat for marine sponges due to the periodical exposure to air and, consequently, varying conditions of temperature and irradiance (including UV radiation). Also, while exposed to air during low tide, the sponges living in the intertidal are not able to filter-feed and, so, may be in need for alternative food sources. Thus, if the photosynthetic activity of photosymbionts is maintained during exposure, it could complement the nutrient uptake particularly of intertidal sponges, and especially in areas where the tidal amplitude is large, resulting in extensive periods of exposure. In addition, intertidal sponge species might also benefit from the possible production of UV-screening substances (e.g., mycosporine-like amino acids) by the photosymbionts.

In Zanzibar (Western Indian Ocean), a large number of sponge species are found both in the intertidal (where the tidal amplitude may be up to 4 m) and subtidal. Their distribution pattern between various habitats is unknown, as is the extent to which these sponges harbour photosymbionts. In this study, 77 sponge species, from five different habitats (including high-amplitude intertidal zones), were examined for the presence or absence of photosynthetic activity. In addition, we evaluated the effects of air exposure on the photosynthetic performance of one intertidal species. A significantly higher frequency of intertidal than subtidal species were found to harbour

Figure 1. Location of the study sites around Zanzibar. The island of Zanzibar (Z) is near Tanzania (T, in grey), northeast of Dar es Salaam (DeS). The sites were: 1 – Matemwe; 2 – Chwaka Bay; 3 – Changuu, also called Prison Island; 4 – Mbwani; 5 – Fumba. See text for further descriptions of the sites.

photosymbionts, and the possible role of photosymbiosis in the former is common discussed.

## 2. Materials and Methods

### *Study site*

This research took place during January and February 2000 in Zanzibar (Western Indian Ocean). The island of Zanzibar is separated from the mainland of East Africa (Tanzania) by a channel that is ca. 30 km wide. The island lies between latitudes 05°40' and 06°30'S and along the longitude 39°E (Fig. 1). Five different sites were chosen: Matemwe – an intertidal lagoon and an adjacent subtidal rocky reef situated on the north-eastern coast; Chwaka Bay – a shallow tidal marsh on the mid-eastern coast of the island; Changuu (also called Prison Island) – a subtidal coral reef located west of Zanzibar Town; Mbwani – a large intertidal sand flat with mangroves, located ca. 5 km south of Zanzibar Town (on the west coast of Zanzibar); Fumba – another intertidal area between the west coast and a small off-shore island. The tidal amplitude was up to 4 m, and occurred around mid-day, during the time of this study.

*Examination of photosynthetic activity*

At each site, all sponges species found were investigated for the presence or absence of photosynthetic activity. Where possible, the sponges were identified in the field based on shape, consistency, texture, colour and habitat. For further analysis and identification, samples were photographed and vouchers were fixed, preserved and deposited in the Zoological Museum of Tel Aviv University. Most subtidal sponges examined grew at depths of 2–5 m below the mean low tide level; in Changuu, however, the study included sponges that grew down to 12 m. The technique of pulse amplitude modulated (PAM) fluorometry, using the portable Diving-PAM fluorometer (Walz, Germany), was used for ascertaining whether a sponge contained photosynthesising organisms. This instrument has been used previously to measure photosynthetic rates of photosymbionts present in other organisms *in situ*, including two Red Sea sponges (Beer and Ilan, 1998). The method enables determinations of quantum yields (Y) of photosystem II as:  $Y = (F_m' - F) / F_m'$ , where F is the chlorophyll fluorescence under a given ambient irradiance, and  $F_m'$  is the maximal fluorescence as measured after the application of a light period lasting for 0.8 s that is saturating for photosynthesis ( $\sim 6000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). When Y was less than 0.1, the sponge was defined as "non-photosynthetic". Higher values of Y indicated photosynthetic activity, and Y values of 0.30–0.55 were typically obtained for those sponges. Measurements were done using a so-called "coral clip" (Walz, Germany), which darkens the sponge surface for a few seconds just before the measurement. Y was first measured on the external surface of the sponge. In cases where no photosynthetic activity was found, the sponges were cut transversally and the possible activity of inner layers was assessed as well. Measurements were performed on cloudless days, between morning and midday.

The photosynthetic response to irradiances was assessed as rapid light curves (RLCs), using the Diving-PAM's internal halogen light source. These curves were generated by first covering the sponge surface with a "coral clip", thereafter Y was immediately determined in darkness, and 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  $Y * \text{the incident photosynthetic active radiation (PAR)}$  as measured by the Diving-PAM's quantum sensor prior to the RLCs. The Diving-PAM's quantum sensor was calibrated in the laboratory against the quantum sensor of a Li-Cor (US) LI-189 quantum meter.

Samples for chlorophyll analysis were taken from those parts of the sponges found to be photosynthetically active. These samples were frozen in plastic vials, kept in darkness, and returned to the laboratory in Israel for analysis. After thawing 3 ml of N,N-dimethylformamide (DMF) was later added to

each vial, and the samples were disintegrated with a homogeniser. The homogenates were kept in darkness at 4°C overnight, after which they were centrifuged, and the supernatants were scanned for absorption in a spectrophotometer at the wavelengths 400–700 nm. The absorbencies at three wavelengths (603, 647, and 663 nm) were used to calculate chlorophyll contents according to the formulae of Moran (1982).

### 3. Results

There was almost no overlap between the 77 species encountered at the five sites examined. Only seven species were found at two different sites, and another one, *Haliclona cymaeformis* (Esper) (formerly *Sigmatocia symbiotica* Bergquist and Tizard), was found at four sites. Sponges of the subtidal were found growing on sand, rocks or reef corals. Intertidal sponges grew either on the sand, directly exposed to the sun, or partially buried in sand or mud (Matemwe, Fumba, Chwaka, and Mbweni), some on rocks in small tidal pools (Matemwe), or within seagrass beds (Mbweni). At the latter site, three *Tedania* species grew on the roots of mangroves, and were shaded from direct sunlight by the trees. The irradiances experienced by sponges growing in the intertidal region otherwise reached values up to 2500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

Of the 77 different species analysed in our survey, a total of 55 (71.5%) were photosynthetic. In all locations, we found a high percentage of sponges containing photosymbionts (64–84%). The lowest frequency (64%) was in Changuu Island; this site differed from the others by having only subtidal sponges (Table 1). The percentage of photosynthetic species encountered in the intertidal areas (n=27) was significantly higher than among the 50 subtidal species examined [ $\chi^2(1)=3.85$ ,  $p<0.05$ ; Fig. 2]. A list of identified species found to be either photosynthetically active or non-active is given in Table 2.

The photosynthetic responses to irradiance were measured on a *Haliclona* sp. from the intertidal area of Chwaka (Fig. 2). The RLCs were performed on sponges either when they were still submerged, just before the tide retreated, or later, after a few hours of exposure to air. As can be seen, the relative ETRs were lower during the period of air exposure. In order to verify to what degree RLCs represent ETRs under natural conditions of irradiance, "point measurements" under ambient light were also performed on the submerged sponges. It was found that, indeed, the ETRs measured by the "point measurements" were close to those found at similar irradiances as produced by the PAM fluorometer in the RLCs (Fig. 3).

Samples for chlorophyll analysis were taken from 34 of the 55 photosynthetic species. On the average, a higher content of chlorophyll per  $\text{cm}^2$  was found in the intertidal photosymbiont-containing species than in the

Table 1. Number of sponge species examined, and the percentage containing photosynthetic organisms, in five different sites of Zanzibar (Western Indian Ocean). Overall 87 sponges were examined, comprising 77 different species. Eight of these species appeared in more than one location (seven species appeared in two locations, and one, *Haliclona cymaeformis* Esper, formerly *Sigmadocia symbiotica* Bergquist and Tizard, appeared at four different sites).

| Site                          | No. of species examined | No. of species found also at other locations | Percentage of photosynthetic species |
|-------------------------------|-------------------------|--|--------------------------------------|
| Mbweni (intertidal)           | 13                      | 2  | 84                                   |
| Fumba (intertidal)            | 11                      | 5  | 82                                   |
| Changuu (subtidal)            | 33                      | 2  | 64                                   |
| Matemwe (inter- and subtidal) | 12                      | 3  | 73                                   |
| Chwaka (inter- and subtidal)  | 18                      | 6  | 72                                   |
| Total number of species       | 77                      | 8  | 71.5                                 |

Table 2. Photosynthetic activity state of identified sponge species from Zanzibar. The species are listed according to the alphabetical order.

| Photosynthetically active species | Photosynthetically non-active species |
|-----------------------------------|---------------------------------------|
| <i>Adocia atra</i>                | <i>Amphimedon</i> sp.                 |
| <i>Aphysina</i> sp.               | <i>Axinella carteri</i>               |
| <i>Axinyssa topsenti</i>          | <i>Callyspongia</i> sp.               |
| <i>Biemna fistulosa</i>           | <i>Haliclona bawiana</i>              |
| <i>Carteriospongia foliascens</i> | <i>Iotrochota</i> sp.                 |
| <i>Cinachyrella</i> sp. 1         | <i>Ircinia</i> sp. 1                  |
| <i>Cinachyrella</i> sp. 2         | <i>Ircinia</i> sp. 2                  |
| <i>Cliona</i> sp.                 | <i>Ircinia</i> sp. 3                  |
| <i>Euryspongia</i> sp.            | <i>Kallypilidion fascigera</i>        |
| <i>Haliclona cymaeformis</i>      | <i>Tethya robusta</i>                 |
| <i>Haliclona debilis</i>          |                                       |
| <i>Haliclona</i> sp. 1            |                                       |
| <i>Haliclona</i> sp. 2            |                                       |
| <i>Hyrtios erecta</i>             |                                       |
| <i>Lendenfeldia dendyi</i>        |                                       |
| <i>Petrosia</i> sp.               |                                       |
| <i>Pseudaxinella coccinea</i>     |                                       |
| <i>Sphaciospongia florida</i>     |                                       |
| <i>Tedania</i> sp. 1              |                                       |
| <i>Tedania</i> sp. 2              |                                       |
| <i>Tedania</i> sp. 3              |                                       |
| <i>Theonella swinhoei</i>         |                                       |
| <i>Xestospongia exigua</i>        |                                       |

Fig. 2.

Figure 2. Percentage of photosynthetic species found in the intertidal (n=27) and subtidal (n=50) environments in five different areas around Zanzibar.

Fig. 4.

Figure 4. Chlorophyll content of 34 different photosymbiont-containing sponge species; 21 from the intertidal and 13 from the subtidal environments. Data are means  $\pm$ SD.

Figure 3. Photosynthetic responses to irradiance [Rapid light curves (RLCs) and point measurements] measured for an intertidal species common to Chwaka. The RLCs were performed on sponges either when they were still submerged, just before the tide retreated (squares, n=5), or later, after few hours of exposure to air (triangles, n=3). The black circle indicates the mean  $\pm$ SD of the point measurements (n=8), which were measured at an average natural irradiance of  $1080 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . PAR – Photosynthetic active radiation; rel. ETR – relative electron transport rate.

subtidal ones (Fig. 4), and this difference was statistically significant, [t(34)=2.21, p<0.05 (t-test)].

#### 4. Discussion

Sponges are usually thought of as submerged organisms, which are highly sensitive to air exposure (Osinga et al., 1999). Under submerged conditions, a major source of nutrition derives from continuously filtering water and, thus, capturing food particles. However, surprisingly many of the shallow water sponges of Zanzibar are intertidal, and among them we found a significantly higher percentage of photosynthetically active species as compared with the subtidal ones. This finding indicates that most of these periodically exposed sponges may have a strong need for the association with photosymbionts. We suggest that this might be partly due to the fact that their filtering capacity becomes interrupted for extended periods during emergence at low tide. The fact that at least one common intertidal species (*Haliclona* sp.) maintained substantial rates of photosynthesis during air exposure supports this suggestion. Thus, we propose here a new role for the photosymbionts in supplying additional organic energy specifically for sponges growing in high-amplitude intertidal zones where they are unable to filter-feed during extended periods of emergence. Also, the higher average chlorophyll content per given surface area in the intertidal photosynthetic species than in the subtidal ones that contained photosymbionts points to a higher concentration of photosymbionts in the former.

In *Haliclona* sp., the photosynthetic response to different irradiances was investigated during both submergence and emergence. While the ETRs were found to decrease at all irradiances tested during the exposure to air (possibly because of the partial desiccation of the sponge tissue, a photoinhibitory effect due to high irradiances and temperature-induced stress), they were still as high as those found for example for a submerged sponge from the Red Sea (Steindler et al., 2000).

Other factors prevailing in the intertidal of tropical environments are high irradiances of PAR and UV. Thus, another possible contribution of photosymbiosis in sponges from the intertidal may be the production of antioxidant substances as a response to high PAR irradiances. An example of this exists in the Mediterranean sponge *Petrosia ficiformis*, that can live both in symbiosis with the cyanobacterium *Aphanocapsa feldmanni* in the light, as well as aposymbiotically in dark caves. For this sponge, Regoli et al. (2000) demonstrated an enhancement of antioxidants in the presence of photosymbionts, as verified by reciprocal transplantation experiments between sites of high and low irradiance. A similar response was found for the coral *Acropora microphthalma*; the activity of antioxidant enzymes decreased with irradiance along a depth gradient (Shick et al., 1995). With regard to UV-induced damage, it is known that the photosymbionts of corals produce mycosporine-like amino acids (Shick et al., 1999), thus providing UV-

protection for both the symbiont and the coral host. Also cyanobacteria, which are common symbionts to marine sponges, are known to produce mycosporine-like amino acids (Gröniger et al., 2000), and there are three reports of mycosporine-like amino acids found in marine sponges (McClintock and Karentz, 1997; Bandaranayake, 1996, 1997). Therefore, it is possible that the higher presence of photosymbiotic sponge species found in the intertidal, is related to a higher necessity for symbiont-derived substances that screen away part of the UV radiation. In addition to this, and the above proposed role of photosymbionts as an additional source of nutrition during air exposure, other adaptational features of intertidal sponges may relate to their cellular and morphological responses to desiccation (Rützler, 1995).

In previous studies, chlorophyll content has been the preferred way to indicate the presence of photosymbionts in sponges (e.g. Wilkinson, 1984). In the present work, PAM fluorometry was found to be a very effective method to discover the presence of photosynthesising organisms in the sponges. It is far quicker than chlorophyll analysis; results are obtained within seconds. Also, it measures actual photosynthetic activity *in situ* (quantum yields or photosynthetic rates as ETRs, cf. Beer et al. 1998) rather than pigment content only (which could also be the result of ingested, non-photosynthesising, phytoplankton). This, and the fact that it can be used non-intrusively for sponge surfaces (where most of the photosymbionts reside), confirms PAM fluorometry as a method of choice when screening for photosymbiotic relationships in invertebrates.

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