



## Light inside sponges

Franz Brümmer<sup>a,\*</sup>, Martin Pfannkuchen<sup>a,1</sup>, Alexander Baltz<sup>a</sup>, Thomas Hauser<sup>a</sup>, Vera Thiel<sup>b</sup>

<sup>a</sup> Biologisches Institut, Abteilung Zoologie Universität Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany

<sup>b</sup> Leibniz-Institute of Marine Sciences, IFM-GEOMAR, Düsternbrooker Weg 20, 24105 Kiel, Germany

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### ABSTRACT

Sponges are the most basal metazoan organisms. As sessile filter feeders in marine or freshwater habitats, they often live in close association with phototrophic microorganisms. Active photosynthesis by the associated microorganisms has been believed to be restricted to the outer tissue portion of the sponge hosts. However, phototrophic microorganisms have also been detected in deeper tissue regions. In many cases they are found around spicules, siliceous skeletal elements of demosponges and hexactinellids. The finding of phototrophic organisms seemingly assembled around spicules led to the hypothesis of a siliceous light transmission system in sponges. The principle ability to conduct light was already shown for sponge derived, explanted spicules. However it was not shown until now, that in deed sponges have a light transmission system, and can harbour photosynthetically active microorganisms in deeper tissue regions. Here we show for the first time, that, as hypothesized 13 year ago, sponge spicules in living specimens transmit light into deeper tissue regions. Our results demonstrate that in opposite to the actual opinion, photosynthetically active microorganisms can also live in deeper tissue regions, and not only directly beneath the surface, when a light transmission system (spicules) is present. Our results show the possibility of massive or globular sponges being supplied with photosynthetic products or pathways throughout their whole body, implying not only a more important role of these endobioses. Our findings also elucidate the in-situ function of a recently more and more interesting biomaterial, which is unique not only for its mechanical, electrical and optical properties. Biosilica is of special interest for the possibility to produce it enzymatically under environmental conditions.

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### 1. Introduction

Sponges (phylum Porifera) are the phylogenetically oldest, extant metazoans. They evolved during the Neoproterozoic, Neoproterozoic-Cambrian period (Xiao et al., 2005) and are grouped into three classes: The Hexactinellida (Reiswig, 2006) and the Demospongiae (Boury-Esnault, 2006), which compose a siliceous skeleton, as well as the class of Calcarea (Bergquist, 1978), whose skeleton is made of Ca-carbonate. Despite their phylogenetic position at the base of all multicellular organisms, sponges (except calcareous ones) are able to build complex silica structures ranging from microscopic dimensions to macroscopic glass structures of several meters in length and more than 10 mm in diameter (Tabachnick, 2002) (e.g. *Acanthascus (Rhabdocalyptus) dawsoni* (Lambe, 1892), *Monorhaphis chuni* Schulze, 1904). In contrast to other organisms, which deposit bio-silica only template controlled (Perry, 2003), demosponges have the unique ability to synthesize their siliceous skeleton enzymatically (Shimizu et al., 1998). The responsible enzyme, silicatein, was first described in the marine demosponge

*Tethya aurantium* (Cha et al., 1999) and subsequently identified also in other demosponges, most prominently in *Suberites domuncula* (Krasko et al., 2000). Silica spicules are built enzymatically as concentric layers of amorphous silica around a central proteinous filament (Müller et al., 2003). Those skeletal elements are the main taxonomic characters for demosponges and hexactinellids, and might prove to provide vital functions to the sponges. In many cases, spicules have been shown to work in the sponge's scaffolding and play a role in e.g. contraction (Nickel et al., 2006) of the sponges. In rare cases spicules even work in the process of prey capturing (Vacelet et al., 1995). Further, spicules mechanically protect the sponges against predators and enhanced the effect of toxic substances by piercing the enemy's dermal tissues (Hill et al., 2005). The spicules' function extending sheer scaffolding and protection is still subject to ongoing research.

Associations of sponges with cyanobacteria (Steindler et al., 2005) and microalgae have been known for many years (Wilkinson, 1980). These microorganisms may form a mutual symbiosis with their host sponge, i.e., provide their host with fixed carbon and/or nitrogen, while their host allocates essential nutrients and shelter (Wilkinson and Fay, 1979). Additionally, cyanobacteria have been implicated as the producers of secondary metabolites isolated from marine sponges (Unson et al., 1994) with diverse putative ecological functions (e.g. protection against predators and bacterial pathogens).

\* Corresponding author. Tel.: +49 711 685 65083; fax: +49 711 685 65096.

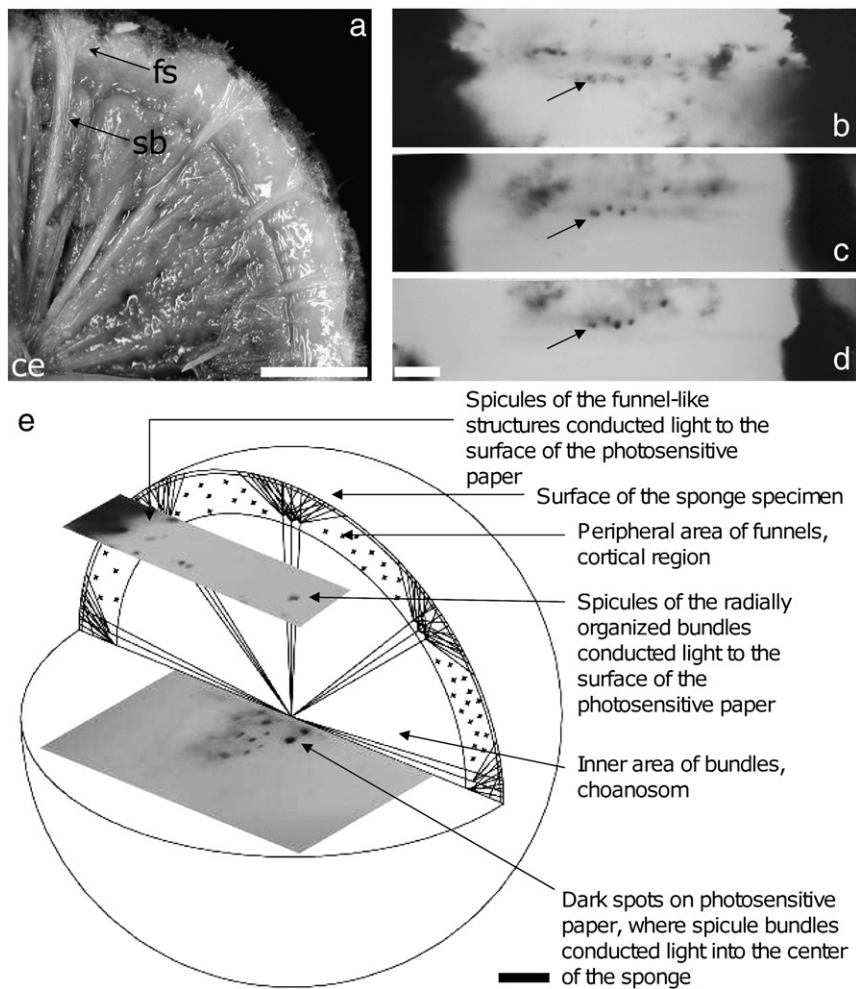
E-mail address: [franz.bruegger@bio.uni-stuttgart.de](mailto:franz.bruegger@bio.uni-stuttgart.de) (F. Brümmer).

<sup>1</sup> These authors contributed equally to this work.

Phototrophic microorganisms in sponges have generally been found in the thin (few mm) outer tissue regions, where enough light energy is available for photosynthesis. Since sponge tissue often is colourful and/or dense and thus not translucent, photosynthetic activity of associated microorganisms was long believed to be restricted to this peripheral region. However, in opposition to this assumption, light dependent, phototrophic endobionts have also been found in deeper tissue regions in several sponges e.g. of the family Tethyidae. Gaino and coworkers observed green microalgae along the sponge's spicules in the inner tissue-parts of the marine sponge *Tethya seychellensis* (Wright, 1881) (Gaino and Sarà, 1994), as well as filamentous cyanobacteria in *T. orphei* Sarà, 1990 (Gaino et al., 2006) in a similar location. In 1994 already the aforementioned authors proposed to test, whether spicules were really used for light transduction into the inner part of the sponges, thus enabling photosynthetic organisms to live in otherwise dark tissue-regions of sponges. Recently, we found phototrophic cyanobacteria of the genus *Synechococcus* (Thiel et al., 2007) associated with the inner tissue of *Tethya aurantium*, where light was also not expected. In agreement with Gaino and coworkers (Gaino and Sarà, 1994), we thus hypothesized the transport of light through spicules into the inner parts of sponges enabling photosynthetic activity of putatively symbiotic cyanobacteria in otherwise dark areas of the specimens. So far, none

of the aforementioned hypotheses had been tested, even though modern biotechnology became interested in sponges as sources of new secondary metabolites as well as biomimetic materials, especially bio-silica (Sarıkaya et al., 2001). In 1996 Cattaneo-Vietti (Cattaneo-Vietti et al., 1996) and coworkers first demonstrated, that isolated spicules derived from the Antarctic sponge *Rossella racovitzae* can transmit light with 635 nm of wavelength *ex-vivo*. Further, Aizenberg (Aizenberg et al., 2004) and coworkers as well as Müller (Müller et al., 2005) and coworkers recently characterized the optical characteristics of isolated silica spicules of the species *Euplectella aspergillum* Owen, 1841 and *Hyalonema sieboldi* Gray, 1835 respectively. The investigated spicules were able to harvest incoming light through terminal, lens-like structures, which increased their light-collecting efficiency. The measured transmission efficiency was 60%. Another peculiarity was the optical filter capacity (Müller et al., 2005): Only light between 615 nm and 1310 nm is transduced. These experiments thus showed the ability of sponge-derived silica structures to transmit light and even filter certain wavelengths when tested *ex-vivo*. However, their function in living sponges was yet to be proved.

Species of the genus *Tethya* are characterized by funnel-like arranged spicules and subsequently radially arranged bundles of spicules which reach from the surface (where the funnels build protruding structures on the surface, called tubercles) to the centre of



**Fig. 1.** Light conduction in *Tethya aurantium*. a: A Cross section of a living *Tethya aurantium*. Funnel like spicule structures (fs) and subsequent spicule bundles (sb) reach from the surface to the centre (ce). b–d: 3 photosensitive paper stripes (5 × 1.5 cm – 5 × 1 cm) where inserted subsequently into the same specimen of *T. aurantium* which was illuminated for 1 min in daylight. In all 3 independent experiments characteristic spots could be reproduced (arrows), where spicule bundles transferred light directly to the surface of the paper. e: Schematic drawing of the experiments, with 2 photosensitive papers inserted in different areas within *T. aurantium*. After 1 min of artificial illumination, the spots on the two papers show light transduction in the funnel like spicule structures in the cortical area, as well as in the subsequent spicule bundles of the choanosomal (inner) area. Bars represent 1 cm.

the spherical sponge body (Sarà, 2002). The funnel like structures in the cortical region of *Tethya aurantium* and the subsequent radial spicule bundles (Fig. 1a,e) are arranged in a way that could allow the harvesting of light with a broad range of incoming wave angles.

To investigate the light conducting properties of siliceous skeletal elements in sponges *in-vivo*, we chose two sympatric species: The aspicular demosponge *Aplysina aerophoba* Nardo, 1843 and the spicule-bearing sponge *Tethya aurantium* (Pallas, 1766). We here proved in living specimens, that spicule-bearing sponges of the species *Tethya aurantium* actually transduce light from their surface along spicule-bundles into deeper otherwise dark tissue regions, while aspicular sponges of the species *Aplysina aerophoba* are dark in deeper tissue-regions.

## 2. Material and Methods

Sponges were collected in the Limski kanal, north of Rovinj, Croatia (Brümmer et al., 2004) in shallow waters. To avoid destruction and other negative effects specimen were collected individually by SCUBA diving (following the CMAS rules for scientific diving) with maximum care and transferred directly to seawater tanks. Contact with air as well as injury was excluded, mechanical as well as chemical stress was reduced to minimum by transporting each specimen in individual wide neck containers series 310 PVC (Kautex Textron GmbH & Co. KG, Bonn, Germany). Sampling by SCUBA diving was carried out following the CMAS rules for scientific diving. No decompression dives were performed. In our experiments, for each species, 5 living, healthy and actively pumping specimen were used.

Photosensitive paper, grade hard (Agfa-Gevaert N.V, Mortsel, Belgium) was inserted into the sponge bodies under red light conditions using specially designed leading-forceps.

The sharpened, thin leading-device guaranteed least tissue-damage and at the same time tightened tissue contact. The insertion wounds were thus as tight as possible which minimized side illumination. The prepared specimens were exposed to either natural sunlight or artificial illumination in a water-depth of several cm (comparable to natural conditions) for the given exposure times. Light intensity was measured employing exposure measuring devices (Mikrovolt Integrator, Type MV2, AT, Delta-T Devices Ltd. Cambridge, UK) as well as PAR-detectors (Panlux, electronic 2, Gossen, Germany).

After digitalizing, data analysis for grey value comparison was carried out using the software package ImageJ (Abramoff et al., 2004). Grey values of dark spots (Fig. 1b-d) where spicules ended on the photosensitive paper were compared to grey values of directly light-

exposed paper. In these cases specimens as well as reference papers were illuminated with photoactive radiation of  $0.01784722 \text{ mmol/mm}^2\text{s}$  (measured with the PAR-detector).

## 3. Results

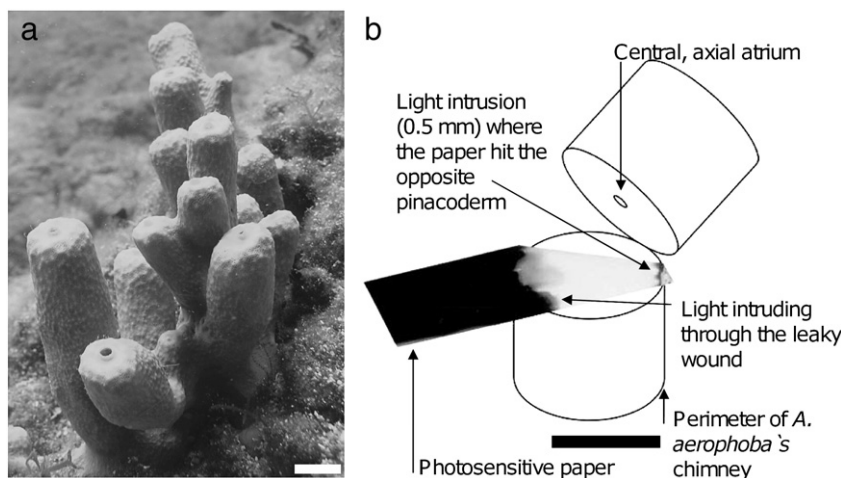
For the tests of factual light transduction through spicules we implanted photosensitive paper into living sponges of the species *T. aurantium* (Fig. 1e) and exposed the specimens to light. After development of the photographic paper we reproducibly found dark spots, where illuminated spicule-bundles reached the photographic paper. As expected, the dark spots were sharp and precise (Fig. 1b-d). They represented illuminated spicule bundles that ended directly on the photosensitive paper. Beyond these concise spots, only little diffuse light was detected. Grey values of the dark spots resulting from spicule bundles ending directly on the photosensitive paper corresponded to up to 0.17% of the applied photoactive radiation (2 - 12% of the natural light exposure).

Concise dark spots in the cortical region (where only short spicules belonging to the funnel like structures end on the photosensitive paper) indicate the light transduction through the funnel-like structures (Fig. 1e). This light is subsequently passed down to the subsequent spicule bundles which again produce concise dark spots if ending on the photosensitive paper (Fig. 1e).

To test whether the aspicular sponge *Aplysina aerophoba* (Fig. 2a) would, as predicted, be unable to transduce light into deeper tissue parts, we performed the same experiment in living specimen of *A. aerophoba*. The photosensitive paper revealed no light within the deeper tissue regions of this aspicular demosponge even after 20-fold longer light-exposure in comparison to *T. aurantium* (Fig. 2b). Only in the leaky wound area and in the contact area of the photosensitive paper and the intact pinacoderm (opposite to the insertion site) some light intruded into the peripheral millimetres. Hence we could detect light intrusion of up to 0.2 mm through the sponge's intact pinacoderm. While the lower detection limit of our setup was  $0.0036 \text{ mmol/m}^2$  and during a 20 min illumination  $21,42 \text{ mmol/m}^2$  were applied, this means, that less than 0.016% of the incoming light is transferred deeper than 0.2 mm into *A. aerophoba*.

## 4. Discussion

In the case of *Tethya aurantium*, silica spicules actually transduce photo-active radiation into living sponges. This is shown by the detection of light inside *Tethya aurantium* on photosensitive paper.



**Fig. 2.** No light conduction in *Aplysina aerophoba*. a: *Aplysina aerophoba* in its environment. b: Schematic drawing of the experimental setup for *A. aerophoba*. As the photosensitive paper indicates, during a 20 minutes illumination, no light is detectably transferred into the aspicular sponge *A. aerophoba*. (For the experiments the chimneys were not cut open, but rather the photosensitive paper was inserted into intact tissue like described.). Bars represent 1 cm.

Since no light could be detected inside the aspicular demosponge *Aplysina aerophoba*, spicules are a necessary prerequisite for the transduction of light into deeper tissue parts of (pigmented) sponges.

The spicule bundles of *Tethya aurantium* are arranged of a large number of single spicules, which are much shorter (several hundred  $\mu\text{m}$ ), than the whole bundle (several cm). That means the detected light inside the sponges is transferred through the spicule bundle and is at the same time transmitted from the outer spicules to the subsequent more central spicules. This at the same time explains the finding of phototrophic organisms along the spicule bundles, because light will leave the spicule bundles while being transferred between the subsequent single spicules. While transmitting light into the sponge body, the silica spicules in living *Tethya aurantium* specimens enable the growth and photosynthetic activity of putatively symbiotic microalgae in the most inner part of the sponge body. These observations do perfectly fit with the observation of photosynthetic organisms, situated around spicule bundles (Gaino and Sarà, 1994) within Tethyidae. Based on a detected natural irradiation of approximately  $771 \mu\text{mol m}^{-2}\text{s}^{-1}$  at the sampling site (in 5 m depth),  $1.3 \mu\text{mol m}^{-2}\text{s}^{-1}$  of PAR will reach the end of the spicule bundles in the innermost part of the sponge in situ. Growth of cyanobacteria (Mullineaux and Emlyn-Jones, 2005) as well as algae (Gupta and Agrawal, 2006) has been shown at very low light intensities of only  $2 \mu\text{mol m}^{-2}\text{s}^{-1}$  already. Thus active photosynthesis and growth of low light adapted cyanobacteria and algae is enabled.

So we now finally proved the hypothesized “possible light conducting system“ of Gaino and coworkers (Gaino and Sarà, 1994) from 1994. This light conducting system allows not only flat or crust like growing sponges to harbour photosynthetically active organisms like already proposed (Taylor et al., 2007), but also enables rather thick and even globular growth forms of sponges (like *T. aurantium*) to harbour phototrophic microorganisms in deeper tissue regions. That way the tissue volume, that possibly benefits from photosynthetic metabolisms is enlarged, allowing also globular or massive morphologies.

## 5. Conclusions

We proved in living specimen, that siliceous skeletal elements of sponges, the spicules, are used as light conductors. They transport photoactive radiation into deep tissue areas of spicule bearing sponges like *T. aurantium*. This finding finally answers the long discussed question on the possible function of spicules in sponges as light conductors. On the basis of this proof, now the function of associated and phototrophic organisms in deep tissue regions of sponges can be investigated with the prerequisite of light being available. In contrast to this, aspicular sponges are not transferring light into deeper tissue regions, which raises new questions on the function of associated phototrophic organisms in inner, thus dark areas of those sponges.

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