USING MICROSENSORS TO MEASURE SPONGE PHYSIOLOGY

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ABSTRACT

Microsensors effectively measure a wide variety of parameters such as chemical concentrations, temperature, light levels and flow. Based on successful investigations of microscale phenomena in sponges we conclude that microsensors are a promising tool for sponge science. We would like to encourage their wider use in sponge physiology (*e.g.* to measure respiration, pumping activity and its regulation), and of the metabolism of associated microorganisms (*e.g.* photosynthesis, nitrification, sulfate reduction). We describe our experiences with liquid ion exchange microsensors, Clarke-type microsensors and microoptodes to provide recommendations for their use on sponges. Possible problems include: 1. sponge texture impeding sensor insertion (due to a strong cortex or spongin); 2. with endolithic or encrusting sponges a sensor can break upon touching the underlying substrate; 3. respiration/photosynthesis measurements may be biased by adhering or fouling plant/animal tissue; 4. contraction/expansion of sponge tissue altering the reference depth during measurements; 5. pumping/different flow regimes causing strong variation of data; 6. ruptured cells may influence chemical properties under investigation; 7. heterogeneity of the sponge body can result in patchiness of data. Some problems can either be overcome by adjustments to the equipment (1, 2) or by preparing the investigated sponge for the intended study (1, 3, 4). Other problems require great care in the recording process and good knowledge of adequate replication (5, 6, 7).

KEY WORDS

Microsensors, method, physiology, metabolism, Porifera.

INTRODUCTION

Microsensors have been developed and improved over the last 20 years, and a wide variety of sensor types are now available (KÜHL & REVSBECH, 2001 and references therein; REVSBECH, 2001). Microsensors most commonly used for biological approaches use electrochemical or optical principles. More recent sensor design takes advantage of the metabolism of live microorganisms encapsulated in the sensor tip (microbiosensors; KÜHL & REVSBECH, 2001).

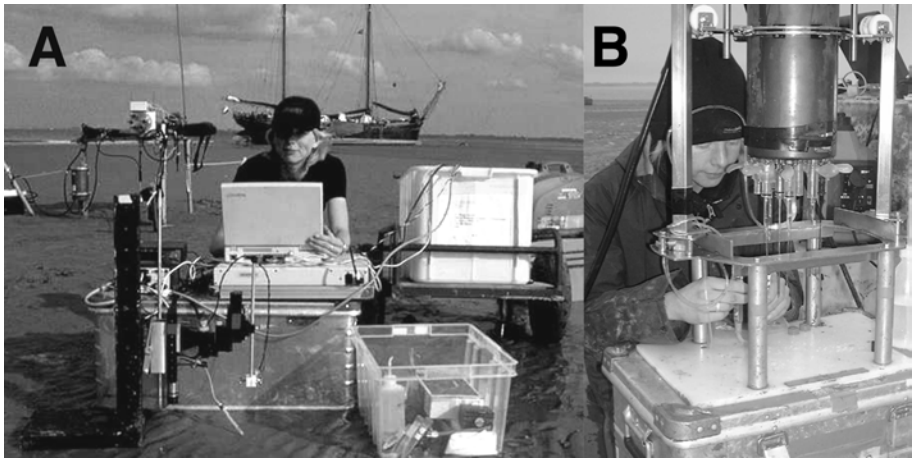

Fig. 1. Application of microsensors during field work. I. DOHRMANN from the Max Planck Institute for Marine Microbiology, Bremen, Germany collecting data in the sediment in the German Wadden Sea. **A,** Data collection with the normal laboratory setup. A microsensor is fixed to an extension and attached to a weighted stand. The sensor is driven into the sediment with a motorized micromanipulator, which is controlled from the computer (use of photograph with friendly permission from E. Walpersdorf). **B,** Calibration of an array of sensors attached to a remotely controlled lander system (use of photograph with friendly permission from P. Bird).

Microsensors have a spatial resolution of ≤ 100 μm (KÜHL & REVSBECH, 2001) and can be moved vertically or at an angle in 1 μm increments with manually operated or motor-driven micromanipulators. Because of the small scale, signal acquisition is comparatively accurate, fast and stable (*e.g.* GATTI *et al.,* 2002). Hence, microsensors are powerful tools, which can be used in environments, in which mini- or macrosensors create an unacceptable level of disturbance.

Microsensors have primarily been developed and employed to investigate boundary layer phenomena, physical and chemical properties of sediment systems, algal and microbial mats and biofilms (*e.g.* REVSBECH & JØRGENSEN, 1983; GLUD *et al.,* 1992; KÜHL *et al.,* 1994, 1996; DE BEER *et al.,* 1997; PLOUG *et al.,* 1999; BOUDREAU & JØRGENSEN, 2001; REVSBECH, 2001). More recently they have been applied in studies on selected species of large protozoans (*e.g.* foraminifera: REVSBECH & JØRGENSEN, 1986; RINK *et al.,* 1998; KÖHLER-RINK & KÜHL, 2000, 2001) and invertebrates (corals: REVSBECH & JØRGENSEN, 1986; KÜHL *et al.,* 1995; DE BEER *et al.,* 2000; AL-HORANI, 2002; sponges and bivalves: GATTI *et al.,* 2002; HOFFMANN, 2003). Difficulties with microsensor work on invertebrates have occasionally been noted but not exhaustively discussed (*e.g.* REVSBECH & JØRGENSEN, 1986). In this article we summarise our combined experiences in order to help other researchers avoid common problems, which are frequently encountered when using this technique on sponges or other similar invertebrates. A small selection of further reading is suggested that will elaborate on the development and the technical background of the tool and give examples of pathways of application.

APPLICATIONS

Which sensors are available and what are their properties?

There is a multitude of microsensors at hand including sensors to measure gradients and fluxes of ions or molecules and to describe *e.g.* environments of flow, light and temperature. Sensors presently available are listed in KÜHL & REVSBECH (2001). Lists combine information about the parameter to be measured with detection limits and principles, tip diameters, lifetime, possible interference, respective literature (their Tabs. 8.1 to 8.3) and commercial providers (their Tab. 8.4). Microsensors can be grouped as electrochemical (including liquid ion exchange sensors = LIX and gas sensors such as the Clarke-type O_2 sensor), optical (including O_2 microoptodes), microbiological sensors and diffusivity and flow sensors (KÜHL & REVSBECH, 2001). Microsensors are technically superior to conventional sensors because of their low disturbance effects, their extremely fine resolution levels and their rapid response (*e.g.* GATTI *et al.,* 2002).

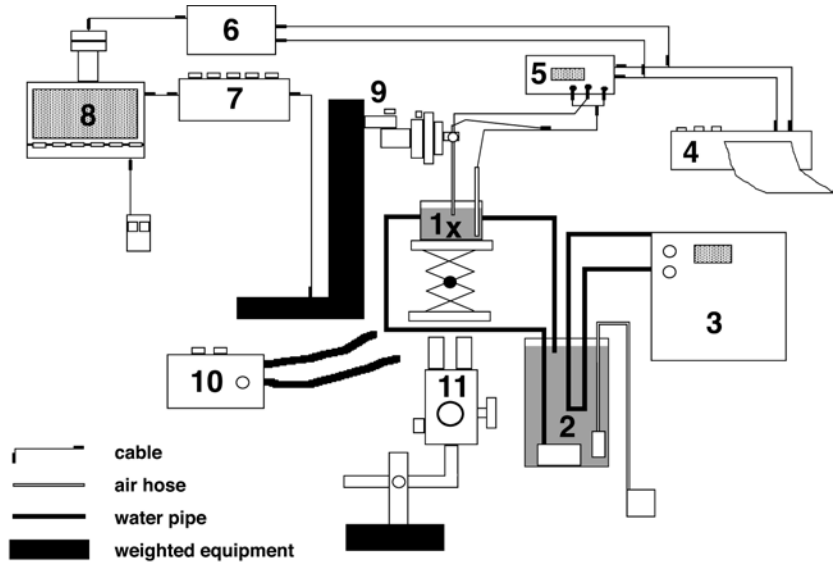

Fig. 2. Commonly used laboratory setup for measurements with liquid iron exchange microsensors (LIX). **1,** Flow chamber containing the sponge (x), on a height-adjustable table. **2,** Water reservoir with water pump which transports the water to the flow chamber and with a bubble stone attached to an air pump. **3,** Computer driven temperature control, with water circulating through pipes leading through the reservoir. **4,** Strip chart recorder as backup for data recording and for visual control of data. **5,** Voltmeter with attached microsensor (left) and reference electrode (right) positioned in the flow chamber. **6,** data acquisition box. **7,** Oriel box which controls the micromanipulator. **8,** Laptop computer for data recording. **9,** Micromanipulator with attached microsensor. **10,** Light source lighting the sample during positioning of sensor tip. **11**, Stereoscope adjusted for viewing the sample. Equipment 3-8 and 10 are also connected to electricity. Cables are not drawn. Equipment drawn with black boxes is weighted down and is thus bulky and heavy.

Getting started

Even though microsensors can be bought through commercial providers (KÜHL & REVSBECH, 2001), the present authors strongly recommend initial training at an established microsensor laboratory. Having achieved a good level of confidence, measurements can be conducted elsewhere, even in the field and in extreme environments (Fig. 1A; *e.g.* GLUD *et al.,* 1994, 1999). Fieldwork generally requires a motor-driven lander system, *i.e.* an adjustable frame in which the sensors are fixed and which can be placed over the studied object (Fig. 1B; *e.g.* WENZHÖFER *et al.,* 2001). However, transporting microsensor equipment may be costly. Many pieces are weighed down for stability and are thus very heavy. Even without extra weight the equipment is bulky. Depending on the specific investigation, a typical laboratory setup may include a flow chamber with a pump and a water reservoir with air supply, a converted stereoscope to monitor the positioning of the sensor, a meter displaying data, a strip-chart recorder, a micromanipulator to move the sensor, a data acquisition box connected to a computer and all connecting and power supply cables (*e.g.* for measurements with LIX sensors, Fig. 2). Extra equipment may include a light source with or without shutter system, a lightmeter, a height-adjustable table for positioning the object and temperature control. The equipment will easily cover 1.5 m length of a laboratory table (*e.g.* for measurements with Clarke-type sensors, Fig. 3A). A further example for the setup with microoptrodes is given in GATTI *et al.* (2002).

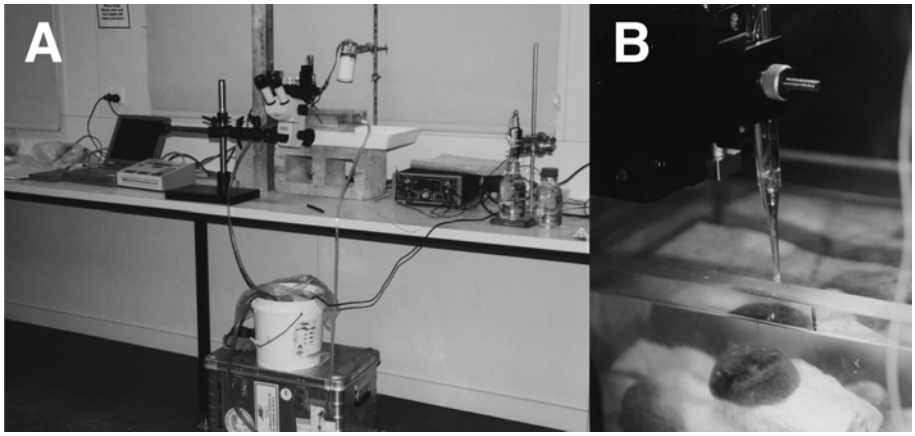

Fig. 3. Commonly used laboratory setup for measurements with Clarke-type oxygen sensors (Photographs by C. Schönberg). **A,** The equipment will take up much bench space. **B,** View of a Clarke-type oxygen sensor fixed in the micromanipulator and positioned on the surface of a graft of *Cliona orientalis*.

For many measurements knowledge of the original pH, salinity and temperature of the reservoir water is essential. Using such background information together with published conversion tables, diffusion and saturation coefficients can be obtained, which are necessary to calculate fluxes (*e.g.* REVSBECH & JØRGENSEN, 1983; GLUD *et al.,* 1992; GUNDERSEN *et al.,* 1998; SCHULZ & ZABEL, 2000). Some measurements related to photosynthesis have to be conducted in a darkened room where accidental

light penetration has to be prevented. Microsensor equipment is sensitive to vibrations and is best set up in a room with as little traffic as possible.

How can microsensors be applied in sponges?

There are two possible approaches: within the sponge tissue and on/near the sponge surface (including measurements in canals). To date the following microsensor types have successfully been applied: in the tissue – oxygen microoptodes for *Suberites domuncula* (GATTI *et al.,* 2002), Clarke-type oxygen sensors for *Geodia barretti* and symbiont-bearing *Cliona* spp. (HOFFMANN, 2003) and calcium and pH LIX sensors for *Cliona celata* and *Halichondria* sp. (SCHÖNBERG, unpubl. data); on the sponge surface or in the surrounding water - oxygen microoptodes for *Cinachyra antarctica* and *Stylocordyla borealis* (GATTI *et al.,* 2002) and Clarke-type oxygen sensors (*Cliona viridis*, *Cliona nigricans* and *Cliona orientalis*: SCHÖNBERG *et al.,* unpubl. data; *Geodia barretti*, HOFFMANN, 2003). These studies investigated oxygen concentration within sponge tissue, oxygen concentration in the tissue of a sponge rich in associated bacteria, chemical indication of bioerosion activitiy in an eroding and a non-eroding sponge species, respiration rates of Antarctic and a Mediterranean sponge and oxygen production in three sponge-zooxanthella associations.

A wide range of further studies could be conducted with microsensors, *e.g.*:

- respiration/metabolism studies with O_2- and CO_2-optodes
- studies on the pumping activity with flow microsensors (which can be inserted into exhalant openings)
- studies focusing on the relationships between sponges and associated microbial organisms using various chemosensors such as Clarke-type oxygen sensors (photosynthesis), ammonium, nitrate and nitrite sensors (nitrification, denitrification) or H_2S sensors (sulphate reduction)
- studying regulatory processes in comparison with the surrounding water using chemoelectrical sensors such as *e.g.* for calcium levels and pH.

In contrast to a macrosensor, a microsensor can be inserted into the sponge *in vivo* without significantly harming it: Sponges show little reaction to the sensor tip breaking the sponge surface as long as the shaft is made reasonably thin (≤ 200 µm in diameter). Even if a comparatively thick sensor is inserted several mm deep into a sponge and the sponge reacts with contraction in the close vicinity of the sensor (in a radius of +/- 0.5 cm), normal activity resumes again after 10 to 60 min after the sensor is removed (papillae reopening in *C. celata*, SCHÖNBERG unpubl. data; similar observations were made for *S. domuncula*, which exhibits normal relaxation after about 15 min, GATTI *et al.,* 2002). Measured profiles through sponge tissue will reveal whether investigated parameters occur in a gradient, in patches, or are evenly distributed throughout the sponge tissue. The use of micromanipulators can resolve such profiles in 1 µm increments, however, larger increments are usually employed. Increment-lengths previously used in sponge-related studies are 50 and 100 µm (HOFFMANN, 2003; SCHÖNBERG *et al.,* unpubl. data).

Fluxes into and out of the sponge can be followed by profiles measured away from the sponge surface. Sensors fastened to micromanipulators can be adjusted to follow various angles, which helps with the sometimes curving or uneven surface of

sponges (Fig. 3B; measurements have to be taken in a line perpendicular to the surface).

PROBLEMS AND SOLUTIONS

Some general problems inherent to the method were discussed by KÜHL & REVSBECH (2001) and by GATTI *et al.* (2002). Many sources of trouble are widely known, and will not be discussed in detail. They include data drift (*e.g.* in sensors in which the membrane has not been allowed to dry properly), short life-time in some sensors (*e.g.* LIX sensors: a few days), and noise in the data recording *e.g.* caused by strong vibrations in and near the laboratory or humid, warm air. The latter inconveniences will occasionally occur regardless of precautions, but will be less frequent with increasing experience. We will thereby focus on typical problems in microsensor studies on sponges. For a more detailed discussion of technical difficulties, please refer to GATTI *et al.* (2002). Another helpful source of summarised information for treating general problems with microsensors and an introduction to their principles and use is the 1996 product catalogue of Fluka Chemie AG, Industriestr. 25, Buchs, CH-9470, Switzerland; http://www.sigma-aldrich.com/Brands/Fluka___Riedel_Home.html).

1. Insertion into tissue: problems caused by texture

When inserting microsensors into dense materials, any horizontal tension or shear force on the electrode tip must be avoided. The largest risk occurs when the sensor tip is merely inserted into the tissue, *i.e.* with the thinnest part of the tip. The examined sponge specimen should be well secured. During profiling, even the slightest touch on the experimental setup can cause breakage. With due care, however, profiles over 1 cm into the sponge tissue can be obtained without damage to the electrode (HOFFMANN, 2003). Additional protection of sensor tips can be acquired by mounting the sensors inside standard 1 ml syringes (GATTI *et al.,* 2002).

Insertion of microsensors into live sponge tissue can be strongly impeded by sponge tissue texture. Two main texture-related problems may arise: many sponges possess a pronounced cortex formed by densely packed spicules; other sponges may have a high content of spongin. While some cortices simply defy insertion and will damage the tip (HOFFMANN, 2003), elasticity of the collageneous spongin prevents insertion, because it does not rupture easily. A sensor tip lowered onto the sponge will cause a dimple in the tissue or dislocate the sponge, but will not readily penetrate. In sponges with an average spongin content, this may lead to the impression that the sensor is inserted deeply into the sponge, when in fact the surface is stretched or the sensor has only partially sunk in. In sponges with a high spongin content the sensor tip will be gradually worn away, but no penetration will be achieved.

Even though the fine sensor tip is usually quite flexible and can be pushed through particles, some sponges present too much textural resistance and some alternative approaches are needed. They include 1) insertion through inhalant and exhalant openings, facilitating more direct access to the softer tissue underneath, 2) carefully cutting the cortical layer with a scalpel or piercing with a canula and sliding

the sensor in through the cut, or 3) the use of wider, more stable sensor tips, which are commonly used in studies in which sensors are driven into sediments.

However, when inserting a sensor through canal system openings there is the risk of measuring in wide canals underneath and not in the sponge tissue (see below). The second approach of cutting the surface potentially bears some risk as well by the greater damage to the sponge compared to when only a microsensor is used. This is especially difficult to avoid in sponges with a high content of spongin, which are best omitted from studies. Substantial tissue damage likely affects measured parameters. Surface cutting was previously used in coral studies where especially in *Favia* sp. copious amounts of mucus were secreted, which affected the insertion properties and the response of the sensor (KÜHL *et al.,* 1995). In contrast, no effect was observed when piercing the surface of the demosponge *G. barretti* with a canula (HOFFMANN, 2003). Thin sensors are difficult to fill with electrolyte, but making the membrane in wide sensor tips is not easy, because leakage occurs before the membrane cures. Moreover, wide sensors are usually slower in response. Nevertheless, they are less likely to break and have greater longevity. For each study it should therefore carefully and individually decided, which alternative approach may work best.

2. Insertion into tissue of endolithic or encrusting sponges: risk of damaging the sensor when touching the substrate

Some problems are caused by the sponges' substrate affinity or growth form. Thinly encrusting sponges may not offer much room to measure profiles through the tissue layer. The progress of the sensor cannot adequately be monitored when it is within the tissue and eventually the tip will make contact with the hard substrate material, which occasionally causes damage to the tip. The same risk occurs during measurements in endolithic sponges, *i.e.* either bioeroding or nestling sponges. Many sponges incorporate foreign particles in their tissue, which can have a similar effect to encountering the hard substrate inhabited by the sponge. If particles are large, they may break the sensor tip. However, small particles can be pushed aside by the sensor. Many sensor tips are flexible enough to survive a slight collision, especially at acute angle.

Hitting the substrate with the sensor usually produces an unexpected change of data caused by mechanical disturbance of the sensor membrane. In all cases the sensor should be re-calibrated after each profile to ascertain whether the sensor is undamaged and still in range with the calibration done before the measurement. If the calibration range is changed the sensor tip needs to be checked under the microscope for evidence of breakage. If it appears to be undamaged, the sensor may exhibit a drift. In any case further use of such a sensor should be avoided and a new sensor should be used. This procedure is adequate to insure reliable measurements with comparatively cheap, less complicated sensors. For sensors, which are costly and difficult to make, preventing damage of the tip is more important. Pre-study crossections of spare organisms may establish the average tissue layer thickness so that profiles can be terminated before reaching the substrate. Sometimes it is advisable to pre-gauge the depth of the sponge tissue with a fine surgical needle in close vicinity to the area to be measured to acquire an estimate how far a sensor can be inserted before touching the background (BIRD, pers. comm.). Subsequent

measurements should not be conducted in the same spot, and a period of adjustment should be allowed for the sponge after the insertion (see 4. below).

3. Sponges with epi- or endobionts: contamination by foreign physiology

Attached or neighbouring organisms may cause contamination of the data. Sponges growing on the surface of plants or sponges fouled by microorganisms may present severe problems. Under different light intensities, oxygen consumption and production levels will vary. Careful checks under the microscope are necessary to ascertain whether the investigated material is contaminated. When measuring in sponges growing on algae, foreign material should be removed if possible and the sponge should be allowed to heal.

Sponges may be covered by fouling microorganisms, especially in stress situations such as after transport and in aquaria conditions. Some species will slough superficial tissue when heavily fouled (*e.g. Halichondria panicea*, BARTHEL & WOLFRATH 1989; *C. nigricans* and *C. orientalis*, SCHÖNBERG, unpubl. data). Freshly moulted surfaces will be comparatively clean of unwanted microbes and offer ideal conditions for measurements.

In contrast, a large variety of endosymbionts may strongly influence the range of data, but are part of the physiology of the association and should not be excluded during the measurements. Comparing measurements in light and dark will establish the different roles of the partners when endosymbionts are photoautotrophs. Presently no method is available to distinguish between respiration rates of sponges and their endobiotic heterotrophs. Moreover, heterogeneous distribution of associated microorganisms may present a problem introducing patchiness. In this case repetitive analyses are necessary to establish the link between altered physiology and microorganism densities.

4. Contraction and expansion: changing reference depth

External stimuli can cause sponges to contract (*e.g.* EMSON, 1966). This is an undesirable effect during microsensor measurements as it introduces noise and because the surface is the reference to any profile measured (all of which also occurs in corals: KÜHL *et al.,* 1995). Ideally the surface should be in the same state of relaxation before and after the measurement. For 1-point measurements in the sponge tissue, measurements should be taken after the sponge has been allowed to relax (GATTI *et al.,* 2002). When profiling in the tissue, it is usually wise to slightly touch the sponge until it contracts, so changes will be minimal. Sometimes the sponge will expand again even though it has a sensor inserted in its tissue (*C. celata*, SCHÖNBERG, unpubl. data). Not much can be done about that, except to reduce measurement periods. When measuring away from the surface it is usually better not to disturb the sponge more than necessary and only lightly touch it with the tip for the first measurement, which will rarely cause contraction (*C. celata*, *C. viridis*, *C. nigricans*, *Halichondria* sp., SCHÖNBERG, unpubl. data). Adequate replication will minimise noise caused by the sponge's behaviour.

5. Sponge pumping and flow regime: the problem with highly variable data

Measurements in the water column above the sponge can be influenced by strong currents created by the sponge's exhalant stream. Small oscillations of data

will suddenly become large and will make measurements of chemical gradients very difficult (*C. viridis*, *C. nigricans*, SCHÖNBERG *et al.,* unpubl. data). Sometimes this effect also occurs at inhalant pores. Measurements will have to be repeated above the sponge surface further away from the openings.

GATTI *et al.* (2002) showed that oxygen concentration in sponge tissue is strongly dependent on the external flow regime around the sponge. For correct interpretation of microelectrode oxygen data obtained within the sponge tissue, sponge pumping rates and external flow velocities around the sponge should be measured at the same time.

6. Rupturing cells: changing chemical signals

Inserting a sensor into specimens of the bioeroding sponge *C. celata* resulted in sudden peaks of lowered pH, possibly due to cell damage. Similar, but weaker peaks were also observed, in the non-eroding sponge *Halichondria* sp. The investigator can somewhat control this effect by choosing between sensor tip morphologies: sensor tips with a bevelled, oblique tip will be more likely to cut into cells, whereas tips with openings perpendicular to the axis are more likely to pass between cells. Moreover, a period of time should be allowed to establish data stabilisation before recording (also after a dark period in photosynthesis measurements: REVSBECH & JØRGENSEN, 1983).

7. Canal system: problems of heterogeneous organisms

REVSBECH *at al.* (1981) and REVSBECH & JØRGENSEN (1983) noted that gas bubbles can form in sediment-microorganism systems, which can cause heterogeneity of data, resulting in an underestimation of photosynthetic rates. A similar problem applies to sponges as their tissues are heterogeneous with distinct tissue layers and the canal system. Chemical concentrations in the canal water are different from those in the sponge tissue, and both are strongly dependent on the metabolic activity of the sponge (HOFFMANN, 2003). In small sponges with high pumping activity, concentrations in canal water may even resemble that of the surrounding medium. With this in mind, unexpected variation in the acquired data can often be explained. Microsensors enable examination of this spatial heterogeneity on a micro-scale. To develop knowledge about typical situations or general trends, however, it is necessary to employ repetitive measurements. The need for repetition as basis for extrapolation of data has previously been discussed by KÜHL & REVSBECH (2001).

As a result of our own experiences we encourage sponge scientists to use microsensors to follow specific research interests. Beginners may find this technique tedious and prone to failure. However, microsensors have been successfully used on sponges and offer a large variety of new opportunities to investigate the physiology of sponges and sponge-dwelling endobionts.

A strong cortex can be bypassed through pores or small cuts of the surface. Alternatively, stronger tips may be made, which may be sturdy enough for insertion through cortices. Sponges rich in spongin should be avoided, as microsensors do not penetrate this material. Care should be taken not to break the sensor's tip on hard substrate the sponge grows on or into. Adhering plant material may bias the results.

Even though microsensors cause minimal disturbance to the sponge, their use may still result in contraction of parts of the sponge tissue. Thus the reference depth taken at the sponge's surface may be altered. Purposely causing a contraction before the onset of measurements may avoid this problem, and keeping measurement periods short will also help. The sensor tip morphology will influence the amount of cell damage caused. Ruptured cells may release chemicals biasing the data. As a consequence, measurements should only be taken when values re-stabilise. Sponges are heterogeneous. Values taken from the tissue may differ from those taken in the canal system or other tissue regions. However, each profile measured in a living system is a result of complex biological and physical interactions at that point of time. Each microsensor application should be considered as a unique set of data, which offers new insights into sponge physiology and ecology.

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