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Sponges in coral reefs

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INTRODUCTION

Sponges are sessile aquatic metazoans, bounded by pinacoderm and containing choanocyte chambers. Choanocytes generate a waterflow from small ostia, through an incurrent and excurrent aquiferous system to larger oscula. Most sponges are massive (crust-, cushion-, fan-, tree- or cup-shape) without distinct symmetry. The mesohyle (between pinacoderm and choanoderm) contains a variety of cell types, collagen and related products and, usually, an inorganic skeleton of silicon dioxide or calcium carbonate. Sexually produced larvae are mostly free-swimming and fundamental to the distribution of the adults. All sponges are active filter feeders, and some use symbioses with bacteria and algae to supplement their energy requirements. Many species are tolerant to epi- and endobiotic organisms (for recent summaries on sponge biology see: Fry, 1970 and Brien *et al.*, 1973; for terminology see: Borojević *et al.*, 1968).

Sponges are an important component of all coral reef communities. Their biomass and range of ecological tolerance frequently exceeds that of the reef-building coral species (Fig. 1a, 1b). They cause considerable impact on their environment by effectively filtering large quantities of water (Reiswig, 1917a, b), by destroying the reef framework (Goreau and Hartman, 1963; Rützler, 1975), by competition for space (Goreau and Hartman, 1966; Rützler, 1970, 1975; Sarà, 1970; Glynn, 1973) and by serving as food source and shelter for numerous fishes and invertebrates (Randall and Hartman, 1968; Tyler and Böhlke, 1972; Rützler, 1976). Nevertheless, due to taxonomical problems and to difficulties in quantitative assessment, quantitative studies of reef sponges are rare.

Although many ecological sponge studies have been made in non-reef environments most of the techniques for collecting, processing for systematic study, biomass determination and quantitative evaluation can be applied to coral reefs.

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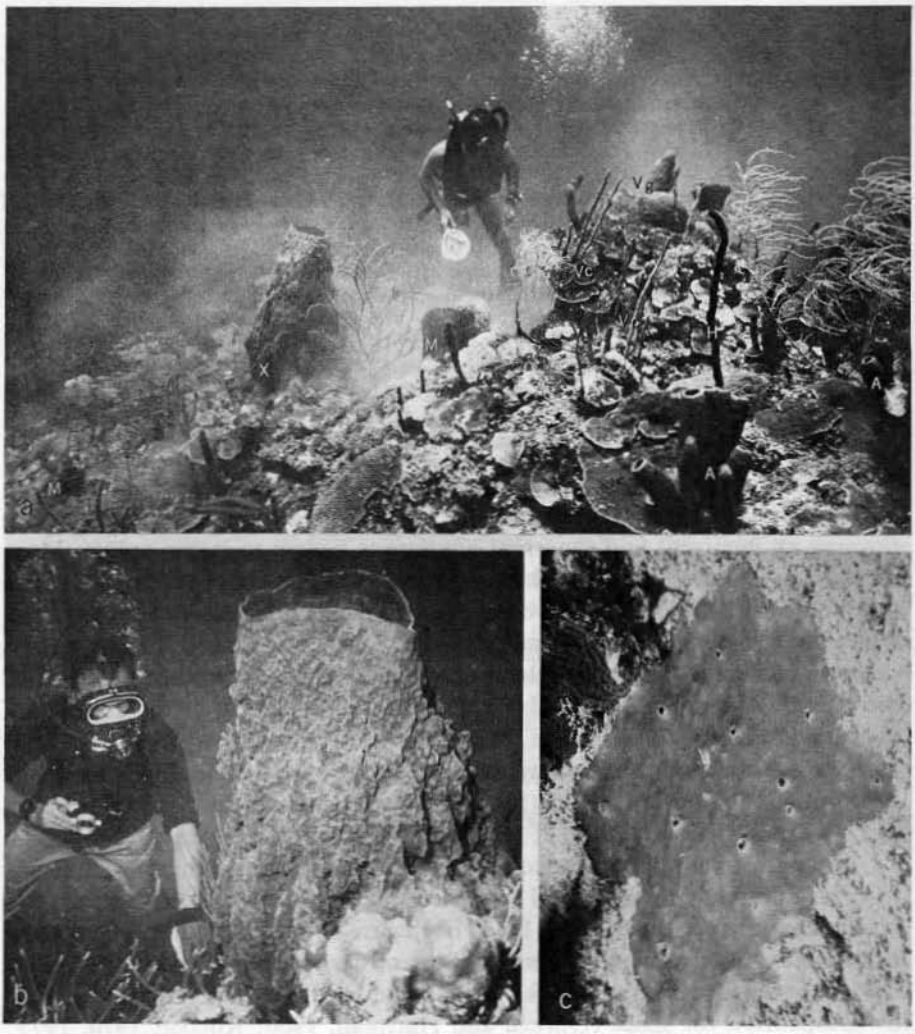

Figure 1
Sponges in a Caribbean coral reef: a, Massive, tubular and whip-shaped specimens at the edge of the fore-reef slope, Carrie Bow Cay, Belize, 20 m deep. A: *Agelas* sp., H: *Haliclona rubens* (Pallas), M: *Mycale* sp., V.c.: *Verongia cauliformis* (Carter), V.g.: *Verongia gigantea* (Hyatt), X: *Xestospongia* sp.; b. Diver with large *Xestospongia* sp., 1.4 m width of field; c, Incrusting-burrowing *Anthosgimella varians* (Duchassaing and Michelotti), 25 cm width of field.

COLLECTING, PRESERVATION AND PROCESSING FOR SYSTEMATIC STUDY

The following is a summary of procedures which can easily be followed by

non-specialized field workers. The resulting data and preparations will not only be the basis for ecological analysis but also an invaluable help for conducting and accelerating identification or systematic study (see also: Laubenfels, 1953; Hartman, 1964; Rubió, 1973).

Habitat data. Most reef surveys will be accomplished by wading, skin or scuba diving, or from submersible vessels. These techniques permit detailed data to be collected by direct observation. The following data should be recorded as completely as possible: date; exact locality (use reliable map and bearings); depth (from mean sea level); substratum (nature and inclination); light (estimate exposure to maximum available light in a given depth); visibility (estimate of suspended materials); exposure (to currents, wave action, and to falling dry-intertidal); sediments (possibility of being buried); community (classify according to predominant organisms); photograph (of habitat and specimen *in situ*).

Specimen data. The entire specimen should be removed from the substratum, including basal membrane (using a sharp knife). Particularly thin incrusting and burrowing forms should be taken with the substratum (using a rock hammer, hammer and chisel). Leave in fresh seawater until ready for fixation. Record the following data: shape (e.g. incrusting, massive; amorphous, ramose, cylindrical, tubiform, vasiform); size (surface area covered, diameter, height); colour (use colour chart, if possible[2]); consistency (e.g. hard brittle, soft elastic, compressible; note mucus production if present); surface (texture; structures like conuli, dilated subsurface channels, embedded sediments); apertures (distribution and size of expanded oscula and pori); photographs (colour, total views and close-ups of surface details—submerged in pan of clean seawater, or in air after removal of excess water).

Fixation. Specimens are fixed (separately) in 10 per cent formalin-seawater (concentrated [37–40 per cent] formaldehyde solution: seawater = 1:9). To neutralize and buffer add 20 g Methenamine ($C_6H_{12}N_4$) to each litre of final solution. To insure good fixation for histological purposes representative slices (about 2 cm^3, including some surface area) should be cut from large specimens and fixed separately. Large specimens can be air-dried after a small portion has been fixed. For the study of sponges with well-developed spongin skeleton, it is useful to cut a similar slice (about 1 cm thick) before fixation and let it macerate in fresh water (repeated rinsing) and dry. This will facilitate later observation of the skeleton architecture. Formalin–seawater is the best all-purpose fixative for marine sponges but it can be replaced by others for specific purposes. Not before 2 days and not after 3 weeks the specimens should be transferred into 75 per cent ethyl alcohol (at least 1 change). Prolonged preservation of sponges in formalin frequently causes maceration of the tissue. Notes should be made

2. A useful and inexpensive colour chart is published by the Royal Horticultural Society, London.

of any colour changes of the fixing and preservation fluids due to exudations from the sponges. With each specimen a water- and alcohol-proof label must be enclosed, showing, specimen number (matched with data sheet); possibly a descriptive field name; locality and depth; collector and date collected; remarks, if applicable (colour change, etc.).

Thick sections. These serve for microscopic study of the skeleton architecture (Figs. 2a, 2b), i.e. the three-dimensional structure of the spongin fibre network, the position of spicules in relation to each other, to the fibre network or to other morphological features (e.g. ectosome, choanosome differentiation). The

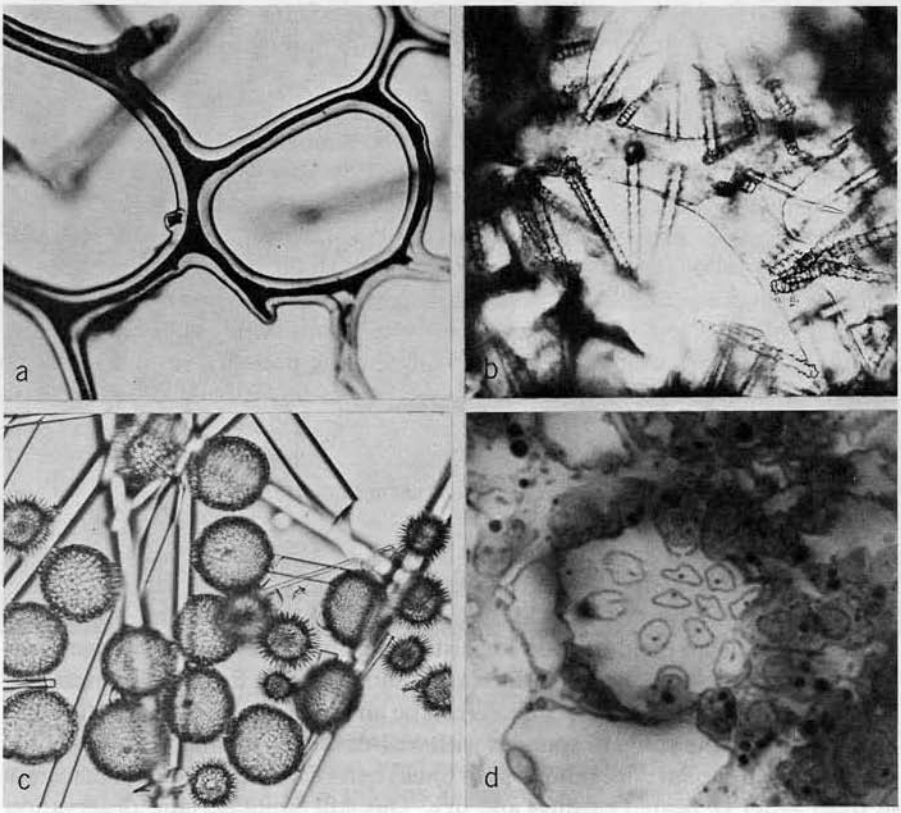

Figure 2
Microscope preparations for systematic study: a, Spongin fibres cleaned from cellular tissue (maceration) of *Verongia fistularis* (Pallas), 1.2 mm width of field; b, Thick section (200 µm) of *Agelas conifera* (Schmidt) showing arrangement of spicules, partially embedded in spongin fibres 500 µm width of field; c, Isolated spicules of *Geodia neptuni* (Sollas), 300 µm width of field; d, Polished thin section of *Cliona lampa* Laubenfels showing choanocyte chamber, safranin-crystal violet stain, 60 µm width of field.

following steps should be taken: transfer a representative piece of the sponge into 96 per cent ethyl alcohol for hardening. Cut with razor blade several slices perpendicular to the surface, as thin as possible (0.2–0.5 mm is usually possible and sufficient). Even thicker sections are permissible (and sometimes necessary) when macerated spongin skeletons are cut. Make sure both ectosomal and choanosomal portions are present on the section. Then make several tangential sections from the surface down to the choanosome. In some species the ectosome detaches easily and can be peeled off with fine pointed forceps. Stain in basic fuchsin or safranin dissolved in 96 per cent ethyl alcohol (10 seconds to several minutes). Solution can be up to saturation, depending on how readily the sections accept the stain. Stain and subsequent dehydrating and clearing fluids can be kept in small petri dishes. Transfer of sections can be done by spatula or forceps. Dehydrate in two changes, one of 96 per cent and the other of 100 per cent ethyl alcohol (30 seconds minimum in each). Observe under stereo microscope the extent to which stain is washed out. If section is understained go back to fuchsin and extend staining time; if overstained extend washing time in 96 per cent alcohol. Dehydrate and clear in saturated solution of phenol in xylene. Transfer to pure xylene and change once. Mount on slide with Canada balsam or similar medium. Thick irregular sections do not hold the cover glass parallel to the slide and balsam tends to run out. This can be avoided by cutting strips of cardboard or short lengths of nylon fishing line (thickness adjusted to section) to support the cover glass. Use small lead weights to press down the cover slips during drying (in oven at 37°C). A label should show specimen number and locality. Near the sections (outside cover glass) the cutting direction can be marked: $\perp$ perpendicular, $\emptyset$ tangential.

Spicule mounts. Whereas the thick sections serve mainly for higher classification of sponges, study of the type, shape and size of isolated spicules (Fig. 2c) is important for species determination (except in those groups where proper spicules are lacking). For temporary mounts, fragments of the sponge (from the ectosomal as well as the choanosomal region) are placed on a microscope slide and a few drops of sodium hypochlorite are added. After disintegration of the soft parts a cover slip is added. For permanent mounts take the following steps: dissolve soft parts of the sponge fragments in a test tube using cold sodium hypochlorite or boiling concentrated nitric acid (except for calcareous sponges). Wash spicules with tap water by filling the test tube and shaking. Await settling of spicules on the bottom (at least 1 hour) or accelerate this process by centrifuging. Decant carefully. Wash spicules twice in 96 per cent alcohol (as above, settling time at least 30 minutes). Decant carefully. Shake and pour sediment on microscope slide. Allow to dry, then add a few drops of xylene, mounting medium, cover slip and label.

Histological sections. These are routinely used for the study of choanocytes and choanocyte chambers, the location of very small microscleres, spongin traces, reproductive cells and, of course, all other histological observations. The