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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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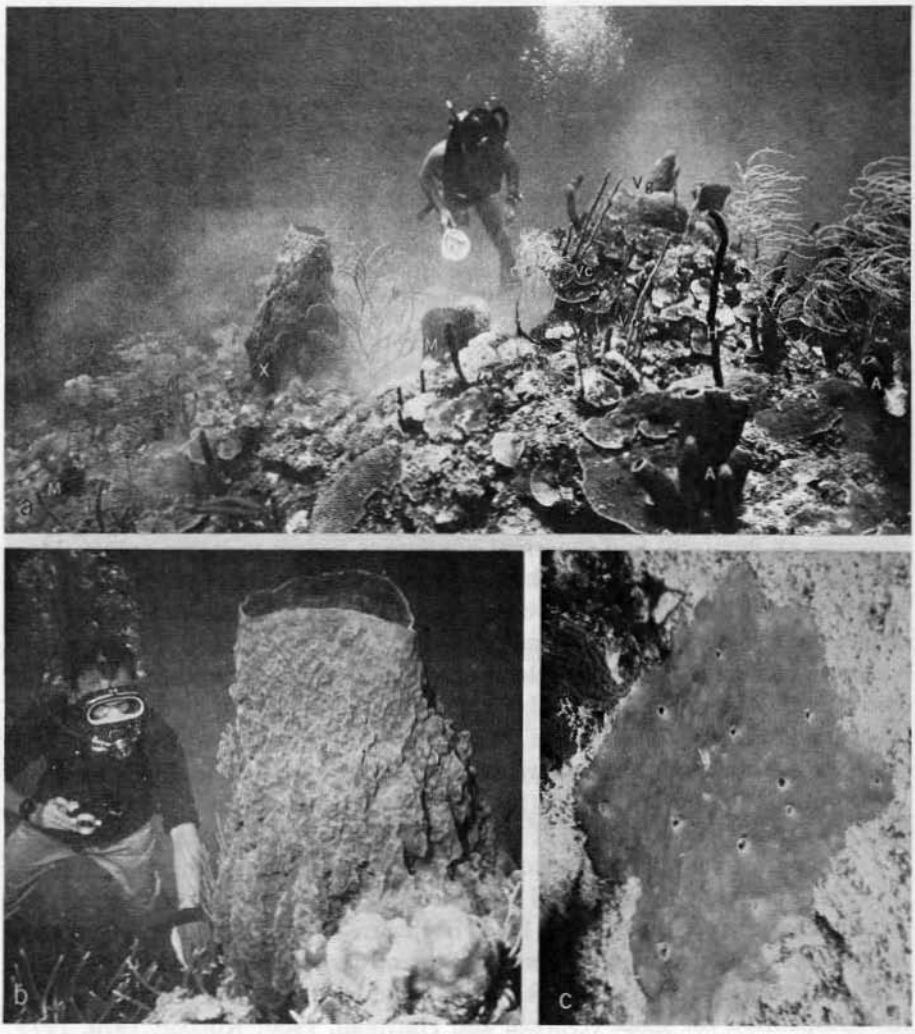

Figure 1
Sponges in a Caribbean coral reef: a, Massive, tubular and whip-shaped specimens at the edge of the fore-reef slope, Carrie Bow Cay, Belize, 20 m deep. A: *Agelas* sp., H: *Haliclona rubens* (Pallas), M: *Mycale* sp., V.c.: *Verongia cauliformis* (Carter), V.g.: *Verongia gigantea* (Hyatt), 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

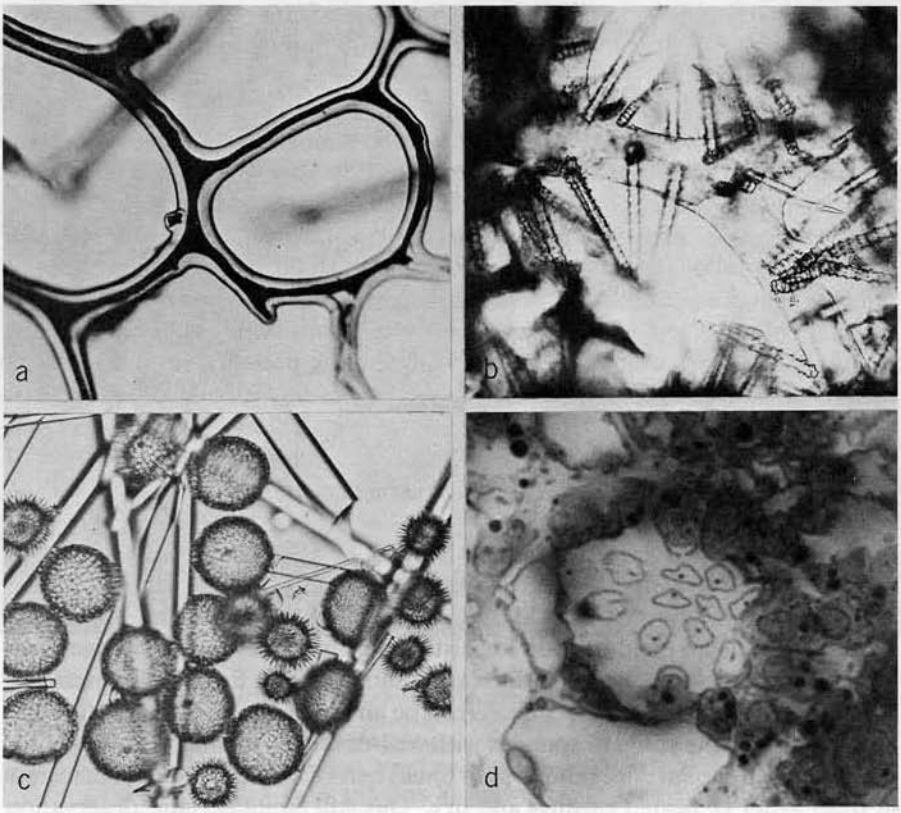

Figure 2
Microscope preparations for systematic study: a, Spongin fibres cleaned from cellular tissue (maceration) of *Verongia fistularis* (Pallas), 1.2 mm width of field; b, Thick section (200 µm) of *Agelas conifera* (Schmidt) showing arrangement of spicules, partially embedded in spongin fibres 500 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

techniques are similar to those which can be found in any general manual or textbook of histological and staining methods. Some special practices are worth pointing out. Calcareous sponges which are usually packed with relatively large spicules can be easily decalcified in 5 per cent nitric acid with subsequent neutralization in 5 per cent sodium sulphate solution (24 hours) and rinsing in tap water (24 hours). The same should be done with all sponges which have large amounts of calcareous particles incorporated in tissue or spongin fibres. The substratum of burrowing (boring) sponges must be removed in the same way: to keep the sponge from falling apart, preliminary embedding in 12 per cent gelatine and decalcification in a cool place are advantageous. Gelatine embedding is useful for all sponges to be cut with a cryostat microtome. An excellent embedding medium for sectioning at room temperature is polyester wax (N. W. Riser, personal communication). Mallory's triple stain is well-suited for routine purposes and for detection of small traces of spongin.

Wet-ground and polished thick and thin sections of epoxy embedded material can be effectively used when demineralization is not desirable and serial sections are not needed (Fig. 2d). The methods are the same as applied to palaeontological preparations, except that tissue is dehydrated in a graded alcohol series and propylene oxide before embedding in epoxy resin. Toluidin blue or, for a double stain, safranin followed by crystal violet (1 per cent aqueous solution, 5–10 mins. at 60°C) give excellent staining results (see also: Rützler, 1974).

DETERMINATION OF BIOMASS

Several methods have been used to express the quantitative importance of sponges within a community. Each has its merits in the context of a particular study and no absolute value judgments are possible. Detailed description of the method used is important to make the results repeatable. Sarà (1966) pointed out two most desirable attributes of a quantitative method, ease of application under difficult field conditions and suitability for statistical elaboration.

Number of individuals. Poriferologists generally agree that a sponge mass bounded by pinacocytes is an individual rather than a colony (Hartman and Reiswig, 1973). Nevertheless, counts of specimens alone are not very useful from a quantitative point of view. The size range, even of sexually mature individuals within a given species can amount to two orders of magnitude (Figs. 1b, 1c).

Surface area. This measure has been used in various ways. Laborel and Vacelet (1958) adopted and modified the Braun–Blanquet phytosociology method where surface area covered by organisms is estimated in percent of the sample square to evaluate abundance. The values obtained were combined with an

index of sociability which also distinguishes whether one and the same area coverage is caused by many small or one large individual. Vasseur (1964) employed this method for different taxa, including sponges and introduced designations for reduced vitality and epizoism.

Russ and Rützler (1959), Sarà (1961), Sarà and Siribelli (1960, 1962) and Rützler (1965a, b) used specimen numbers and area of projected surface coverage (in cm^2) to study quantitative distribution of predominantly encrusting Mediterranean sponges. A similar method was employed by Storr (1964) for reef biota in the Bahamas; however, there is but little information on sponges. Wiedenmayer (1977) studied the quantitative distribution of massive and erect sponges near Bimini (Bahamas). He too measured projected surface area but oriented the projection plane to obtain maximum area, depending on the growth form of each species. Surface values have been obtained by Rützler (1965a, b) by counting 1 cm^2 meshes of a overlaying grid drawn on a plexiglas plate or, more accurately, by planimetry of paper traces from projected photographs (Rützler, 1975).

Sarà (1966) introduced a frame counting method by which frequency of occurrence of sponges in a 5 cm grid are recorded. This technique also results in sponge coverage values.

Volume. When one is dealing with large, irregular massive, tree-shaped, tubular and cup-shaped sponges, the projected surface area becomes an inadequate measure for biomass. For this reason Rützler (1972) determined the displacement volume of sponges sampled in a reef off Nossi-Bé (Madagascar). Drip-dried specimens were submerged in a container filled with seawater to the level of an overflow tube. the amount of displaced water could be determined using a graduated cylinder. This method, however, requires collecting of all specimens to be measured. The displacement technique can also be used to estimate the internal space system, available to an endobiotic fauna, in relation to the sponge tissue volume (Rützler, 1976). Whole sponges were enclosed tightly in thin plastic foil and submerged to measure total volume. From this the value of the displacement volume without wrapping can be subtracted to give the interstitial space volume.

A convenient volumetric field method was used by Reiswig (1973, 1974) in a Jamaica reef. Specimen size was determined by measuring the three axes of each specimen *in situ*. The net volume, excluding atrial cavity, where present, was estimated by applying the values of a predetermined regression curve.

In a comparable manner Dayton *et al.* (1974) converted linear measurements, taken from photographs of Antarctic sponges into wet weight values. The length (or diameter) to wet weight regression had been pre-determined for each species.

Weight and calorific content. Although a common practice in ecological studies, weight measurements have not been much used for sponges. The procedures are tedious and require equipment which is not usually available in

the field. As with displacement volume determinations, specimens have to be removed from the habitat. The method, however, if properly used, gives the most accurate measure of biomass.

Russ and Rützler (1959) supplemented area coverage with wet-weight data but did not compensate for the varying amount of skeleton material (spicules) in different species. Rützler (1975) determined wet- and dry-weight for 8 species of burrowing sponges and related these data to the size of their papillar fields, a measure that can be readily determined *in situ*. In this example the weight data were important to compensate for specific differences in penetration depth and burrowing patterns. The spicule to soft tissue ratio was fairly constant among all species and could be neglected. Dry-weights proved to be slightly more repeatable than wet-weights.

If accurate biomass comparison of species with very different skeleton contents is required, ash-free weights, or another measure that excludes hard tissue, e.g. organic carbon content, must be determined. Calculation of energy budgets may require measurement of calorific content of tissues.

Randall and Hartman (1968) presented data on the relation of ash content to organic matter in 18 species of sponges which are frequently eaten by West Indian fishes. The content ranges from 0.5 per cent in *Chondrosia* to as much as 70 per cent in *Anthosigmella*.

For comparative study of metabolism in 3 species of Jamaican reef sponges Reiswig (1973, 1974) determined weight and calorific content of tissues. Dry weights were obtained by drying to constant weight at 105°C, ash weights by combustion at 500°–510°C for 2 hours. Corrections were applied for water loss from siliceous spicules (Paine, 1964). The results (Reiswig, 1973) showed relatively low ash values of 31 per cent for *Mycale* (Poecilosclerida) and 34 per cent for *Verongia* (Dictyoceratida) as opposed to 62 per cent for *Tethya* (Hadromerida). Calorific values per ml sponge, obtained by microbomb calorimetry were high in *Verongia* (407 cal/ml), only about half of that amount in *Mycale* (200 cal/ml) and *Tethya* (214 cal/ml). In *Mycale* over 40 per cent of the calories are contained in skeletal spongin.

Similar parameters (dry weight, ash-free dry weight, calorific content of tissue) were measured by Dayton *et al.* (1974) to quantify the standing crop of an Antarctic sponge community.

Oxidizable carbon is considered a 'realistic measure of the energy stored in a crop' (Strickland and Parsons, 1968). In developing a preliminary model of a coral reef ecosystem, the participants in a recent workshop meeting on Glover's Reef (Belize, Central America: 1971) have agreed to use carbon as the universal measure for biomass and material flow (Macintyre *et al.*, 1974). The meeting also stimulated preliminary determinations of wet-weight- dry-weight: carbon ratios in Caribbean reef sponges (Rützler, unpublished). Specimens of ten species of sponges belonging to six orders were collected in Bermuda. Two samples of each species were cleaned from sediments and symbionts under a stereo microscope. Wet-weight was obtained after draining off interstitial water and quick superficial blotting. Dry-weight was determined

after drying to constant weight at 95°C over calcium sulphate. Carbon content was measured using a Coleman Model 33 carbon-hydrogen analyser standardized with tartaric acid. Samples of *Dysidea* had been previously decalcified to exclude inorganic carbon from sediment carbonates included in its fibres. The results are summarized in Table 1.

TABLE 1. Wet-weight: Dry-weight: Carbon ratios in Bermuda Sponges[a]

Order and species	Dry-weight as percentage of wet-weight	Organic carbon as percentage of dry-weight
Dictyoceratida		
Ircinia cf. *fasciculata* (Pallas)	13.3	27.8
"Ianthella" ardis Laubenfels	19.3	33.5
Dysidea fragilis (Montagu)	22.0	23.6
Haplosclerida		
Haliclona viridis (Duch. & Mich.)	24.1	24.2
Gelliodes ramosa (Carter)	17.1	33.3
Callyspongia vaginalis (Lamarck)	13.8	34.0
Poecilosclerida		
Tedania ignis (Duch. & Mich.)	19.1	28.0
Halichondrida		
Ulosa sp. *("Dysidea crawshayi")*	32.4	21.9
Hadromerida		
Chondrilla nucula Schmidt	16.9	13.7
Spirophorida		
Cinachyra cf. *cavernosa* (Lamarck)	26.9	26.1

[a]. Species allocation following Laubenfels (1950).

QUANTITATIVE SAMPLING METHODS

The ultimate aim of any ecological study is to find cause-effect relationship between environmental parameters (including biotic influences) and distribution patterns of species and individuals (biomass). Each species has developed its own strategies to cope with environmental conditions. The tolerance of a species to factor gradients determines its distributional range (frequency). Approach of optimum conditions is reflected by an increase of individuals or biomass (abundance). The number of coexisting species (diversity) is a measure for environmental complexity (number of available niches).

Problems and methods for the quantitative study of sponge populations are the same as those applying to any other group of sessile organisms. As there is no single best technique, the methods used are generally a compromise between available time and means and required results for a given situation.

Diversity and abundance estimates. Simple distributional principles, as caused by a factor gradient, can be recognized by examining the faunal composition of zones which are defined by different light intensities. For example, Sarà (1961) and Pouliquen (1972) delimited areas of decreasing illumination (e.g.

shaded, obscure and dark) in Mediterranean caves. They listed the sponge species in each zone and determined their relative abundance (e.g. present, rare, abundant, very abundant). Although these data are not suitable for statistical elaborations, they do represent a measure of diversity and permit comparisons with other habitats.

In a similar manner, without having a uniform sample size for statistical treatment, Rützler (1965b) determined the influence of substrata stability on the diversity and growth of a sponge fauna inhabiting the lower surfaces of loose rocks.

The majority of quantitative sponge studies, however, are based on quadrat sampling.

Quadrat size and sampling area. Quadrat size and sampling area are usually not identical. Choice of suitable quadrat size depends on the method of data collecting, the complexity of the environment and the size range and density of the organisms to be studied. If the entire sample is to be removed and brought to the laboratory, if the factor gradients are steep (complex substrata configuration, heterogeneous microclimate), and if the organisms are comparatively small and densely spaced, squares of $\frac{1}{16}$ m^2 (25 × 25 cm) can be used (Russ and Rützler, 1959). For visual census and coarse area coverage estimates involving very large sponges in a homogeneous environment, quadrats of up to 100 m^2 (10 × 10 m) have been considered necessary (Wiedenmayer, 1977). The best suitable sample size has to be found empirically for each application. If the quadrat is too small, distortion in statistical treatment can occur (Ryland, 1973). If it is too large, environmental changes cannot be resolved.

For statistical reasons all sampling units should be of equal size. For applications where this is not possible, a method of conversion based on computer simulation has been introduced (Ryland, 1972). The sum of all samples within a stratum (community) represents the sampling area. Its minimum size can be determined by the species-area curve (Cain, 1938; Riedl, 1953). Another, more recently introduced, method is based on binomial sampling theory (Dennison and Hay, 1967). The minimum proportional presence of a species within the study area must be found empirically. From a graph one can then determine the sampling area required for a desired probability value for collecting efficiency.

Quadrat sampling and evaluation. The phytosociological quadrat method has been applied by Laborel and Vacelet (1958) and Vasseur (1964) for the study of sessile populations (including sponges) in a Mediterranean cave and in Indian Ocean reef habitats. Two coefficients, quantifying abundance and sociability, were estimated *in situ* for a number of species. The data are not statistically usable but serve for recognition and comparison of characteristic plant and animal assemblages and their dependence upon such factors as light, hydrodynamics and silting.

The influence of intertidal exposure, light, water movement and sedimen-

tation on sponge distribution was determined by Russ and Rützler (1959) and Rützler (1965a, 1972) for Mediterranean caves and for an Indian Ocean fringing reef. Transects were selected which represented profiles through factor gradients (e.g. in a cave, from light to dark, from ceiling to bottom). Along these transects, quadrat samples ($\frac{1}{16}$ m^2 or $\frac{1}{4}$ m^2) were marked in fixed distances. Most samples were removed entirely (including burrowed portions of the substratum) and taken to the laboratory for species and biomass determinations. Some quantitative estimates were made *in situ* and from photographs. Where transects were meaningless, random samples had to be chosen within areas of apparently uniform environmental conditions. Sample data were then arranged in groups corresponding to different intensities of a particular parameter. Care was taken to eliminate counteracting factors (e.g. samples representing a light gradient were chosen from vertical substrata with average hydrodynamic exposure, to avoid influences from sediments and excess water-movement). Indices of homogeneity (Riedl, 1953) were calculated to determine faunal similarities between samples assigned to one microclimatic regime, and to compare the community structure of different environments.

Although this method is objective and suitable for statistical treatment, it is tedious and not suitable for large scale application.

With these difficulties in mind Sarà (1966, 1970) introduced a quick quadrat counting method for the quantitative study of Mediterranean shallow-water sponges. A 25 cm × 25 cm frame was subdivided with nylon filament to form a grid of 25 meshes (5 cm × 5 cm). The frame was placed on the substratum and the number of meshes occupied by each species was counted. The frequency of counts is correlated with the surface extension of the individuals. The effect of this method is similar to chain link counts which have been used for coral diversity studies (Porter, 1972). Rützler (1975) adopted the frame counting method for large scale distributional studies of burrowing sponges in Bermuda reefs. It was modified to express estimated coverage values (in cm^2) inside the meshes and calibrated for the surface area-biomass ratio of each species.

SUMMARY AND CONCLUSIONS

Synopsis. Compared with other important reef organisms, sponges have been greatly neglected in quantitative studies. The principal reasons for this are taxonomic problems and difficulties in quantification, due to great variability in shape and size.

Identification of sponge taxa is much facilitated if guidelines for proper preservation, data collecting and micro-anatomical preparation are followed.

Specimen counts have limited quantitative application. Biomass estimates in the field can be based on projected surface area or on measurement of the three main axes of a specimen. It is necessary, however, to correct the data for variations in skeleton contents, or tissue porosity, by submitting subsamples of

each species to laboratory analysis. In this manner, quick and reliable estimates of weight, volume or energy content can be obtained, without need to remove the entire sample.

Quadrat sampling along transects and, at random, with defined zones has been used for quantitative sponge studies in rocky sublittoral and coral reef areas. Quadrat size and sample area vary with size classes and distributional characteristics of the fauna. Phytosociological techniques are best suited to uncover characteristic organism assemblages and interactions. Other, more objective methods, involving counts of species presences, or biomass measurements, can be evaluated statistically and have been applied to reveal relationships between factor complexes and faunal distribution patterns.

Methods adopted for I.M.S.W.E. Reef Project. The current Investigations of Marine Shallow Water Ecosystems (I.M.S.W.E., Smithsonian Institution) Coral Reef Project is conducting quantitative studies on reef biota off Carrie Bow Cay, Belize.

The zoning of the reef complex has been mapped, using a permanent main transect. This transect starts in the *Thalassia* zone of the lagoon and extends, perpendicular to the direction of the barrier reef to the 45 m depth mark on the fore-reef slope. Habitats, characterized by dominant organisms (e.g. *Millepora* zone, *Turbinaria* zone) or geological features (e.g. rubble and pavement zone) have been and are still being mapped and their position fixed in relation to the main transect.

Principal organism assemblages in each habitat are quantified using auxiliary transect counts (crossing habitat boundaries) and random quadrat counts (within habitats). (Figs. 3c, 3d). Chain transects (mainly in coral dominated habitats) are compared with quadrat transects (quadrat positioned every 2 m on the transect). Quadrats vary in size from 0.5 m $\times$ 0.5 m to 2 m $\times$ 2 m, depending on the size classes of organisms. Area coverage of each species (dimension of main axes, for large sponges) is determined *in situ* (Figs. 3a, 3b), subsamples are taken to the laboratory for biomass assessment (wet weight, displacement volume). Substratum complexity is taken into consideration and sample size is corrected for functional surface area (Dahl, 1973).

Each habitat is subdivided into strata, which are defined on the basis of microclimatic conditions and substrata properties. For example, colonized (unburied) lower surfaces of rocks, seagrass rhizomes or leaves, interstitial spaces or burrows in dead coral, sand or rubble are each considered a stratum and sampled separately, after its proportional extension within the habitat had been noted. Volume samples replace surface area counts, where appropriate (e.g. rubble, sand). Interstitial organisms are extracted after anaesthetizing with magnesium chloride or by oxygen depletion (Kirsteuer, 1967).

Environmental parameters applying to each stratum are measured or estimated at the time of sampling. Whenever possible, these factors are monitored or checked over long periods of time to learn about intensity means and extremes.

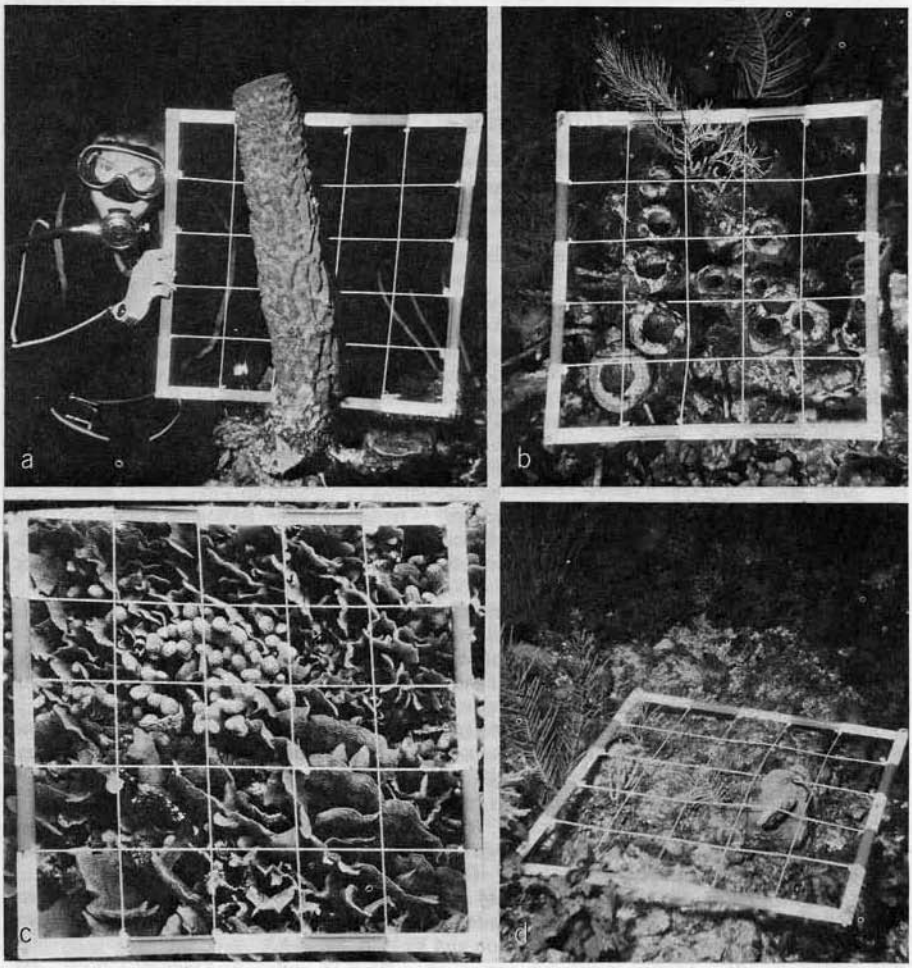

Figure 3
Quantitative methods applied during coral reef study at Carrie Bow Cay, Belize: a, Diver holding 25 × 25 cm frame to record height and width of large tubular *Verongia* sp.; b, Frame on top of tube cluster of *Agelas* sp.; c, Frame overlaying *Agaricia-Porites* patch (spur and groove system) for point counting at the intersections of the filament grid; d, Situation like in c, but frame placed on the pavement with some *Agaricia*, gorgonians and green algae.

A suitable method of multivariate analysis will be applied (R. G. Domey, pers. comm.), as soon as sufficient amount of data has been processed.

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