

Relationships of biological and taxonomic characteristics to chemically mediated bioactivity in Mediterranean littoral sponges

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Abstract. The aim of this study was to detect relationships of bioactive properties (antibacterial, antifungal, antiviral, antimitotic and cytotoxic activities) of Mediterranean littoral sponges with some of their biological characteristics (growth habits, presence of symbiotic or epibiotic organisms, structural defences) and their systematic position. Antimitotic and cytotoxic activities were present in 80.6% and 73.6% of the species respectively, 42.2% of the species showed antibacterial activity, 29.8% were antiviral and 22.5% were antifungal. Only antiviral and antifungal activities were significantly dependent on taxonomical order, being especially important in the Axinellida. Antiviral, antifungal and antibacterial activities predominated in erect forms, and were poorly represented in encrusting forms, which, however, included a higher percentage of cytotoxic and antimitotic species. Nevertheless, only antiviral activity was significantly related to growth habit. All types of activities were significantly dependent on sponge skeletal features: the highest percentages of species with cytotoxic activity were found among horny and siliceous sponges. Also, antiviral and antibacterial activities were better represented in horny sponges. Antibacterial, antifungal and antiviral activities were dependent on the presence/absence of epibionts and seemed to be more efficient as antifouling defences than antimitotic and cytotoxic activities. Only cytotoxic activity was significantly more widespread in species harbouring cyanophyceae. Correspondence analysis revealed that cytotoxic and antimitotic activities were both related to encrusting forms, a siliceous skeleton, occasional epibiosis and the presence of cyanophyceae. Antifungal, antibacterial and antiviral activities were mainly related to erect or globular form, siliceous and horny skeleton and habitual (species-specific) epibiosis.

Introduction

In every latitude examined, sponges have been reported to be one of the marine macrobenthic groups in which the

production of biologically active substances is most widespread (e.g. McClintock 1987, Munro et al. 1989, Blunt et al. 1990, Uriz et al. 1991).

If the production of active metabolites plays a relevant role in species protection, as has been stated by most authors (Coll et al. 1982, Faulkner and Ghiselin 1983, Sammarco et al. 1985, La Barre et al. 1986, Pawlik et al. 1987, 1990, Faulkner 1988, Hay and Fenical 1988, Paul and Van Alstyne 1988), it seems logical that chemical defences have had particular evolutionary success in sessile, surface-dependent and slow-growing organisms like sponges. Similarly, some relationships between production of bioactive substances and certain biological or taxonomical characteristics of the chemically active species could be expected.

Three main roles have traditionally been attributed to the chemically mediated bioactivity in benthic organisms: competition for space (Jackson and Buss 1975, Buss 1976, Sammarco et al. 1983), antifouling defences (Al-Ogily and Knight-Jones 1977, Stoecker 1978, Thompson et al. 1985, Amade et al. 1987, Wahl 1989, Bakus et al. 1990, Sears et al. 1990, Uriz et al. 1991, Wahl and Bagnais 1991) and chemical deterrence (Bakus and Green 1974, Stoecker 1980, Bakus 1981, Paul and Hay 1986, Hay et al. 1987a, b, Paul 1987, Faulkner 1988, Hay and Fenical 1988, Shanks and Graham 1988, Bakus et al. 1989, Steinberg and Paul 1990). Studies focused on sponges, carried out in the Atlantic, Pacific, Indian or Antarctic oceans, have demonstrated ichthyotoxicity (Green 1977, McClintock 1987, Green et al. 1990, Huysecom et al. 1990), cytotoxicity (Blunt et al. 1990), antibacterial activity (Burkholder and Ruetzler 1969, Bergquist and Bedford 1978, Faulkner 1978) and antimicrobial, antitumour or immunomodulatory properties (Amade et al. 1982, Munro et al. 1989, Green et al. 1990), as well as the capacity of sponges to inhibit some biological functions (Thompson et al. 1985). However, only antibacterial, antifungal and ichthyotoxic activities have been studied extensively in sponges of the Mediterranean Sea (Amade et al. 1987, Van der Vyver et al. 1990).

The purpose of this study was to detect a possible association of bioactivity of Mediterranean littoral sponges

(antibacterial, antifungal, antiviral, antimutagenic, and cytotoxic) with some of their biological characteristics (growth habits, the presence of symbiotic or epibiotic organisms, structural defences) and their taxonomical position.

Although the assays of bioactivity were carried out using terrestrial organisms, the activities studied indicated the presence of chemically active organic molecules in sponge extracts which might serve a biological function. The application of multivariate analysis to assess the relationships between activities and biological or taxonomical parameters in a relatively high number of species was an attempt at a statistical approach to the interpretation of the significance of this chemical bioactivity. Nevertheless, the global results of this analysis should be confirmed by experimental procedures, as has been done for sponges from other latitudes (Sullivan et al. 1983, Thompson 1985, Plucer-Rosario 1987, Porter and Target 1988).

Material and methods

Sampling

Sampling was carried out by SCUBA, during which areas 4 m wide and from 0 to 55 m in depth were exhaustively explored. The zones investigated include 27 sampling stations off the Balearic and Columbretes archipelagos (Fig. 1). The following communities or groups of communities, described in Uriz et al. (1991), were considered: photophilic algae (PA); sciaphilic algae (SA); precoralligenous (PC); coralligenous blocks, upper sides (CBU); coralligenous blocks, lower sides (CBL); semi-obscure caves (SOC); *Posidonia oceanica* meadows (PM); and euryhaline and eurythermal lagoons (EEL). The samples yielded a total of 180 specimens belonging to 59 species of Porifera (see Table 1), which represent about 95% of the sponge biomass inhabiting the communities examined.

Biological data

The growth strategies considered in this study (boring, thick encrusting, globular and erect forms) are related to the amount of

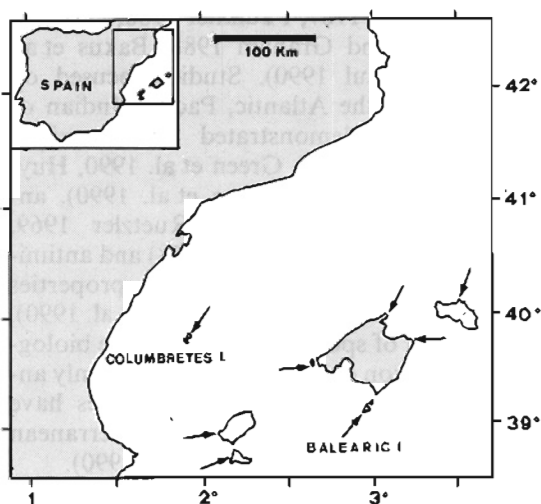


Fig. 1. Location of sponge sampling stations

substratum surface required to maintain a certain sponge biomass. Therefore, body form may be related to the necessity of developing chemical mechanisms of competition for substratum.

Thick encrusting, globular and erect forms were particularly well represented in the samples. Small, thin encrusting sponges, the remaining 5% of sponge biomass, were not taken into consideration because it was not possible to collect the amounts required for the bioassays.

The groups of structural defences considered were related to the chemical nature of the skeleton: siliceous, calcareous, horny, and lacking conspicuous skeletal structures. *Chondrosia reniformis* was included in the horny group, despite the fact that it lacks conspicuous sponge macrofibers, and because of the great amount of fibrillar collagen it possesses (see Table 1).

Data on epibiosis were recorded on every specimen collected, and the species were divided into three categories: 0 = never fouled; 1 = occasionally fouled; 2 = habitually fouled. Data on microsymbiosis came mainly from the literature (Lévi and Lévi 1965, Vacelet 1970, Sarà 1971, Vacelet and Donadey 1977, Wilkinson et al. 1981) and also from microscopic observations.

Assays

Since the aim of this study was to find possible relationships between bioactivity and biological or taxonomical characteristics at the species level, a combination of from two to four specimens of each species was used in the bioassays in order to avoid intraspecific variability due to uncontrolled exogenous (environmental) causes.

Small pieces (2 g wet wt) of various specimens of each species were drained for 1 min, weighed, homogenized together (in the proportion 1 g of sponge per 10 ml of 3:1 methanol/toluene) and centrifuged (Rinehart et al. 1983). Sterile paper discs, 6 mm in diameter, were soaked with 10 µl of the supernatant, air-dried and used in the activity assays.

Antimutagenic tests were performed on leukemic cells of mice (L1210) by determining the percentage inhibition of cell growth. The assays were scored as follows: 0 = no inhibition; 1 = 1 to 25% inhibition; 2 = 26 to 50% inhibition; 3 = 51 to 100% inhibition.

Antibacterial and antifungal activities of the crude organic extracts were determined by the diffusion method (Bergquist and Bedford 1978, Thompson et al. 1985) on cultures of *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. The nutrient-agar plates were incubated overnight at 37°C and zones of inhibition around the discs were measured. The mean value of the inhibition zones for the two microorganisms used to test each type of antimicrobial activity was scored as: 0 = no growth inhibition; 1, 2 and 3 = inhibition zone < 2 mm, between 2 and 4 mm, and > 4 mm wide, respectively.

Cytotoxic tests were performed on kidney cells of monkey (CV-1) incubated for 3 d at 37°C. Cytotoxicity was calculated by determining zones of cell inhibition (Rinehart et al. 1983) and graded as: 0 = not growth inhibition; 1 = inhibition zone to 1 mm; 2 = inhibition zone from 1 to 2 mm; 3 = inhibition zone from 2 to 4 mm.

Antiviral tests were performed with herpes simplex virus, type 1 (HSV) cultured in kidney cells of monkey (CV-1), and with vesicular stomatitis virus (VSV) in kidney cells of hamster (BAK), as described in Schroeder et al. (1981). Mean values of the zones of virus inhibition on the two viruses were scored as for cytotoxic activity.

Control tests with the solvent were performed for every assay.

Numerical methods

Data are presented here in two different ways to highlight complementary information. The percentage of active species (bar charts) gives an idea of how each of the bioactivities is distributed in the sponge group representing a given characteristic. The dependence/independence of these percentages on the biological and taxonomi-

cal parameters was established by means of the G statistic for an $R \times C$ contingency table (Sokal and Rohlf 1979).

In addition, correspondence analysis (Legendre and Legendre 1979), performed on an activities-per-species matrix, integrates the relationships between all the activities and all the biological and taxonomical characteristics considered. These relationships are graphically illustrated here by representing each species by its biological characteristics or taxonomical status.

Results

Incidence of different activities in sponges

Among the 59 species studied only six (*Clathrina cerebrum*, *Crella pulvinar*, *Faciospongia cavernosa*, *Hexadella detritifera*, *Stelligera rigida* and *Stryphnus mucronatus*) did not show any kind of activity (Table 1). Antimitotic and cytotoxic activities were present at various intensities (indices 1, 2 or 3) in 80.6% and 73.6% of the species, respectively. Nevertheless, high cytotoxic activity was more widespread than high antimitotic activity among sponges.

Antibacterial activity was distributed among 42.2% of the species, antiviral in 29.8% and antifungal in 22.5% (Fig. 2).

Relationships between activities

Correspondence analysis (Fig. 3) indicated a clear association between antimitotic and cytotoxic activities. A similar association has been detected in a wider group of benthic organisms including algae, Cnidaria, Tunicata, Echinodermata and Mollusca (Uriz et al. 1991). Antiviral, antibacterial and antifungal activities appeared isolated. The first axis (41.9% of variance explained) was defined by a segregation between antiviral activity and antimitotic and cytotoxic activities. The second component (24.7% of variance) separated the antifungal and antibacterial activities in the positive sector from the antimitotic, cytotoxic and antiviral activities in the negative sector.

Activities and taxonomical groups

Antimitotic and cytotoxic activities were widespread in all orders of the class Demospongiae. They were also present in the order Calcinea of the class Calcarea, although results for the few species tested from this class do not permit generalizations. Both activities, together with the antibacterial, were distributed independently of the taxonomical group considered (Fig. 4). In contrast, antiviral and antifungal activities were significantly dependent ($p < 0.025$ and $p < 0.01$, respectively) on taxonomical order and were especially well represented in the Axinellida. Antifungal activity was also relatively important in the Homosclerophorida and Calcinea, antiviral activity in the Dictyoceratida and antibacterial activity in the Axinellida and Dictyoceratida.

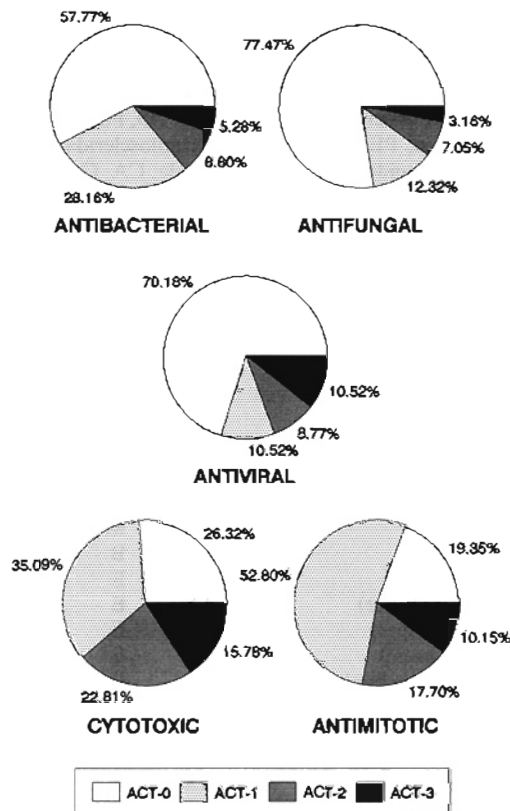


Fig. 2. Percentages of the four activity levels in 59 species of Mediterranean sponges, for the five types of activities tested. ACT-0, no response; ACT-1, low response; ACT-2, medium response; ACT-3, strong response

Certain antimicrobial activities were absent from some orders: antiviral activity did not occur in the Homosclerophorida, Halichondrida, Haplosclerida, Dendroceratida or Calcinea, while antifungal activity did not appear in the Halichondrida or Haplosclerida, and antibacterial activity was absent from the Astrophorida and Homosclerophorida (Fig. 4).

In the correspondence analysis, some species of Axinellida, along with one species each of Homosclerophorida, Calcarea and Hadromerida, were correlated with antifungal and antiviral activities (Fig. 5). Antiviral activity was also associated with some Dictyoceratida and one Poecilosclerida (Fig. 5). The position of antibacterial activity (Fig. 5) was the result of the interaction between species that also exhibit antiviral activity (negative sector of the second axis). Representatives of a broad range of orders, especially the Poecilosclerida, Haplosclerida, Hadromerida, Halichondrida and Dictyoceratida, were placed in the zone of antimitotic and cytotoxic activities.

The only species of the family Dysideidae (*Dysidea avara*) was located closer to *Pleraplysilla spinifera* (order Dendroceratida) than to the remaining Dictyoceratida (Fig. 5). Within the family Thorectidae, species of the genus *Ircinia* Nardo *sensu stricto* tended to occur near the antiviral and antibacterial activities (positive sector of the first axis), while *Ircinia* species of the subgenus *Sarcotragus* Schmidt appeared closer to the cytotoxic and antim-

Table 1. Sponge species tested with reference to systematic order (Ord.), growth habits (Form), skeletal types (Skel.), indices of epibiosis (Epi.), and presence of cyanophyceae (Cyan.) and bacteria (Bact.). Orders: AS, Astrophorida; HO, Homosclerophorida; CH, Chondrosiida; HA, Hadromerida; AX, Axinellida; PO, Poecilosclerida; HL, Halichondrida; PE, Petrosida; HP, Haplosclerida; DY, Dictyoceratida; VE, Verongida; DR, Dendroceratida; CA, Calcinea.

Forms: M, globular; EN, encrusting; B, boring; E, erect. Skeletal types: SI, siliceous; W, without; HOR, horny; CA, calcareous. Indices of epibiosis; 0, never fouled; 1, occasionally fouled; 2, habitually fouled. Activities: BA, antibacterial; FU, antifungal; AM, antimicrobial; AV, antiviral; CT, cytotoxic. See "Materials and methods" for explanation of activity assay scores. Number next to species names correspond to those in Fig. 3 B

| Species | Ord. | Form | Skel. | Epi. | Cyan. | Bact. | BA | FU | AM | AV | CT |
|--|------|------|-------|------|-------|-------|----|----|----|----|----|
| 1 <i>Geodia cydonium</i> (Jameson) | AS | M | SI | 1 | - | - | 0 | 0 | 0 | 0 | 1 |
| 2 <i>Erylus euastrum</i> (Schmidt) | AS | M | SI | 0 | - | - | 0 | 1 | 1 | 1 | 3 |
| 3 <i>Stryphnus mucronatus</i> (Schmidt) | AS | M | SI | 1 | - | - | 0 | 0 | 0 | 0 | 0 |
| 4 <i>Oscarella lobularis</i> (Schmidt) | HO | M | W | 0 | - | B | 0 | 2 | 1 | 0 | 3 |
| 5 <i>Plakortis simplex</i> Schulze | HO | EN | SI | 0 | - | - | 0 | 0 | 1 | 0 | 3 |
| 6 <i>Chondrosia reniformis</i> Nardo | CH | M | HOR | 0 | - | B | 0 | 0 | 1 | 1 | 1 |
| 7 <i>Tethya aurantium</i> (Pallas) | HA | M | SI | 1 | C | - | 1 | 0 | 0 | 0 | 1 |
| 8 <i>Laxosuberites rugosus</i> Schmidt | HA | EN | SI | 0 | - | - | 0 | 0 | 1 | 0 | 1 |
| 9 <i>Spirastrella cunctatrix</i> Schmidt | HA | EN | SI | 0 | - | - | 1 | 0 | 2 | 0 | 3 |
| 10 <i>Cliona viridis</i> (Schmidt) | HA | M | SI | 0 | - | - | 0 | 1 | 1 | 0 | 0 |
| 11 <i>Cliona celata</i> Grant | HA | M | SI | 0 | C | - | 0 | 0 | 1 | 1 | 1 |
| 12 <i>Cliona carteri</i> (Ridley) | HA | B | SI | 0 | - | - | 0 | 0 | 1 | 0 | 1 |
| 13 <i>Axinella</i> sp. | AX | E | SI | 0 | - | - | 1 | 1 | 1 | 3 | 1 |
| 14 <i>Axinella damicornis</i> (Esper) | AX | E | SI | 2 | - | B | 1 | 2 | 3 | 2 | 1 |
| 15 <i>Axinella verrucosa</i> (Esper) | AX | E | SI | 2 | - | - | 3 | 3 | 2 | 2 | 2 |
| 16 <i>Acanthella acuta</i> Schmidt | AX | E | SI | 0 | - | B | 1 | 2 | 1 | 3 | 0 |
| 17 <i>Phakellia rugosa</i> (Bowerbank) | AX | E | SI | 0 | - | - | 1 | 0 | 1 | 0 | 0 |
| 18 <i>Raspaciona aculeata</i> (Johnston) | AX | EN | SI | 0 | - | - | 2 | 2 | 1 | 3 | 1 |
| 19 <i>Rhabderemia minutula</i> (Carter) | AX | EN | SI | 0 | - | - | 0 | 0 | 1 | 0 | 0 |
| 20 <i>Stelligera rigida</i> (Montagu) | AX | E | SI | 0 | - | - | 0 | 0 | 0 | 0 | 0 |
| 21 <i>Agelas oroides</i> (Schmidt) | AX | E | HOR | 0 | - | B | 0 | 0 | 1 | 3 | 1 |
| 22 <i>Phorbas tenacior</i> (Topsent) | PO | EN | SI | 0 | - | - | 0 | 0 | 2 | 0 | 3 |
| 23 <i>Hamigera hamigera</i> (Schmidt) | PO | EN | SI | 0 | - | - | 0 | 1 | 2 | 0 | 2 |
| 24 <i>Mycale contarenii</i> (Martens) | PO | EN | SI | 0 | C | - | 0 | 0 | 1 | 0 | 1 |
| 25 <i>Crella pulvinar</i> (Schmidt) | PO | EN | SI | 0 | - | - | 0 | 0 | 0 | 0 | 0 |
| 26 <i>Crambe crambe</i> (Schmidt) | PO | EN | SI | 0 | C | B | 2 | 1 | 3 | 0 | 3 |
| 27 <i>Clathria oxeifera</i> Ferrer Hernandez | PO | M | SI | 1 | - | - | 0 | 0 | 0 | 1 | 0 |
| 28 <i>Halichondria contorta</i> (Sarà) | HL | EN | SI | 0 | - | - | 1 | 0 | 1 | 0 | 1 |
| 29 <i>Ciocalyptra penicillus</i> Bowerbank | HL | EN | SI | 0 | - | - | 0 | 0 | 0 | 0 | 1 |
| 30 <i>Batzella inops</i> (Vosmaer) | HL | EN | SI | 0 | C | - | 0 | 0 | 1 | 0 | 1 |
| 31 <i>Dictyonella obtusa</i> (Schmidt) | HL | EN | SI | 0 | - | - | 0 | 0 | 1 | 0 | 1 |
| 32 <i>Hymeniacidon sanguinea</i> (Grant) | HL | EN | SI | 1 | C | - | 0 | 0 | 1 | 0 | 1 |
| 33 <i>Hemimycale columella</i> (Bowerbank) | HL | EN | SI | 0 | C | - | 0 | 0 | 3 | 0 | 3 |
| 34 <i>Petrosia ficiformis</i> (Poiret) | PE | M | SI | 1 | C | B | 0 | 0 | 1 | 1 | 2 |
| 35 <i>Haliclona mediterranea</i> Griessinger | HP | M | SI | 0 | - | - | 0 | 0 | 1 | 0 | 0 |
| 36 <i>Reniera cratera</i> Schmidt | HP | M | SI | 1 | C | - | 0 | 0 | 1 | 0 | 0 |
| 37 <i>Reniera fulva</i> Topsent | HP | EN | SI | 0 | - | - | 1 | 0 | 3 | 0 | 3 |
| 38 <i>Reniera mucosa</i> Griessinger | HP | EN | SI | 0 | - | B | 1 | 0 | 3 | 0 | 2 |
| 39 <i>Ircinia dendroides</i> (Schmidt) | DY | M | HOR | 0 | - | B | 1 | 0 | 0 | 3 | 1 |
| 40 <i>Ircinia fasciculata</i> (Pallas) | DY | M | HOR | 1 | C | B | 1 | 0 | 2 | 0 | 2 |
| 41 <i>Sarcotragus muscarum</i> (Schmidt) | DY | M | HOR | 1 | - | B | 1 | 0 | 3 | 0 | 3 |
| 42 <i>Ircinia oros</i> (Schmidt) | DY | M | HOR | 0 | - | B | 2 | 0 | 1 | 3 | 3 |
| 43 <i>Sarcotragus spinosula</i> (Schmidt) | DY | M | HOR | 1 | - | B | 2 | 0 | 1 | 0 | 1 |
| 44 <i>Ircinia variabilis</i> (Schmidt) | DY | M | HOR | 0 | C | B | 3 | 0 | 0 | 2 | 2 |
| 45 <i>Spongia officinalis</i> Linné | DY | M | HOR | 0 | - | B | 0 | 0 | 2 | 1 | 2 |
| 46 <i>Hippospongia communis</i> (Lamarck) | DY | M | HOR | 1 | - | B | 0 | 0 | 1 | 2 | 2 |
| 47 <i>Faciospongia cavernosa</i> (Schmidt) | DY | M | HOR | 1 | - | B | 0 | 0 | 0 | 0 | 0 |
| 48 <i>Cacospongia scalaris</i> Schmidt | DY | M | HOR | 2 | - | B | 1 | 0 | 2 | 2 | 2 |
| 49 <i>Dysidea avara</i> (Schmidt) | DY | M | HOR | 0 | C | B | 1 | 1 | 2 | 0 | 2 |
| 50 <i>Aplysina aerophoba</i> Schmidt | VE | M | HOR | 0 | C | B | 3 | 0 | 1 | 0 | 2 |
| 52 <i>Hexadella racovitzae</i> Topsent | DR | EN | W | 0 | C | - | 1 | 0 | 1 | 0 | 1 |
| 53 <i>Hexadella detritifera</i> Topsent | DR | EN | W | 1 | - | - | 0 | 0 | 0 | 0 | 0 |
| 54 <i>Pleraplysilla spinifera</i> (Schulze) | DR | EN | HOR | 1 | - | B | 2 | 1 | 2 | 0 | 2 |
| 55 <i>Halisarca dujardini</i> Johnston | DR | EN | W | 1 | - | B | 0 | 0 | 1 | 0 | 0 |
| 56 <i>Darwinella australiensis</i> Carter | DR | EN | HOR | 0 | - | - | 0 | 0 | 1 | 0 | 0 |
| 57 <i>Clathrina cerebrum</i> (Haeckel) | CA | M | CA | 0 | - | B | 0 | 0 | 0 | 0 | 0 |
| 58 <i>Clathrina clathrus</i> (Schmidt) | CA | M | CA | 0 | - | B | 1 | 3 | 2 | 0 | 2 |
| 59 <i>Clathrina coriacea</i> (Montagu) | CA | EN | CA | 0 | - | - | 0 | 0 | 1 | 0 | 0 |

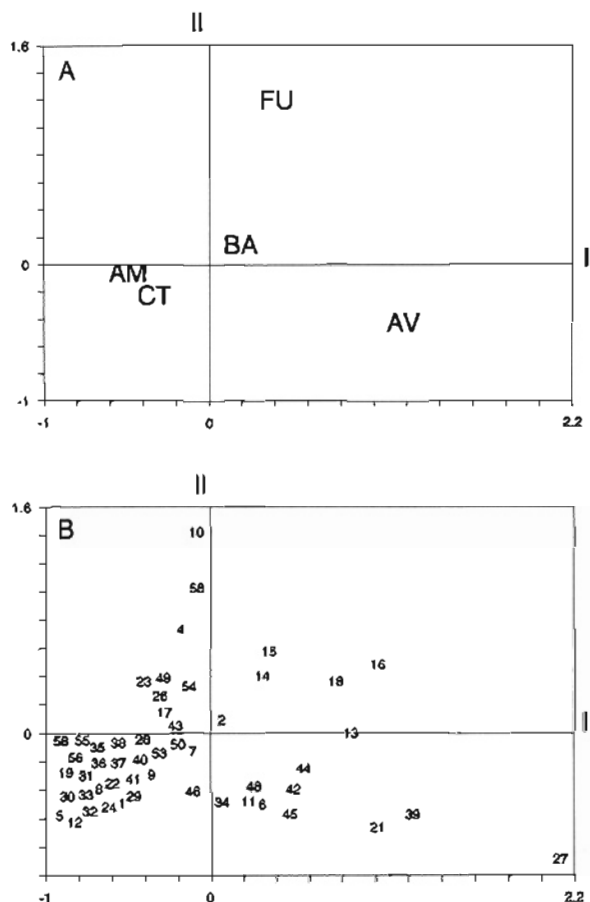


Fig. 3. (A) Distribution of bioactivities in the first two axes of the correspondence analysis. (B) Distribution of species (numbers correspond to those in Table 1). AM: antimitotic; CY: cytotoxic; FU: antifungal; BA: antibacterial; AV: antiviral

itotic activities. Finally, the species *Raspaciona aculeata* and *Agelas oroides* appeared together with the majority of the Axinellida, whereas *Rhabdermia minutula* was closer to the group containing the Poeciloscleroida, Halichondrida and Hadromerida.

Activites and growth strategies

Even though antimitotic and cytotoxic activities were present for every growth strategy considered, both were somewhat better represented in encrusting forms (90.9 and 77.2%, respectively; $n=22$) (Fig. 6). On the other hand, antiviral, antifungal and antibacterial activities predominated in erect forms (55.5, 44.4 and 66.6% respectively; $n=9$), followed by globular forms (44, 20 and 40%, respectively; $n=25$); these activities were poorly represented in encrusting forms. The tests of significance, however, indicated that only antiviral activity was distributed differentially among the different growth forms ($p < 0.001$).

The single boring species tested only showed slight cytotoxic and antimitotic activities (index 1).

Correspondence analysis (Fig. 7A) showed that almost all the species displaying encrusting forms occurred in the sector corresponding to cytotoxic and antimitotic

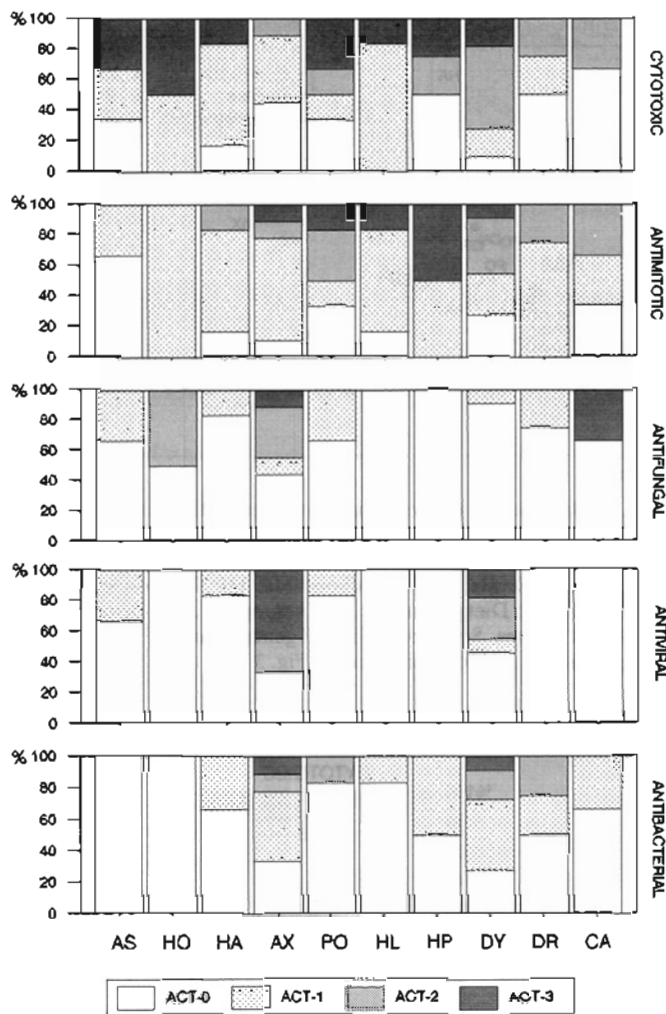


Fig. 4. Percentages of active species in the various orders studied for each type of activity tested. AS: Astrophorida; HO: Homosclerophorida; HA: Hadromerida; AX: Axinellida; PO: Poecilosclerida; HL: Halichondrida; HP: Haplosclerida; DY: Dictyoceratida; DR: Dendroceratida; CA: Calcinea (orders represented by a single species are not shown). Abbreviations of activity levels as in Fig. 2

activities. Globular and erect sponges were related to antifungal and antibacterial activities, while antiviral activity was exclusively related to globular forms. The boring form behaved like encrusting forms.

Relationships between chemical and structural defences

All types of activities were distributed according to skeleton type ($p < 0.001$) (Fig. 8). Activities were not especially higher in sponges lacking skeletons, except for antimitotic activity. The highest percentages of cytotoxic species were found for sponges with horny fibers (86.6%, $n=14$) and with siliceous skeletons (71.5%, $n=37$). Antiviral and antibacterial activities were also better represented in horny sponges (53.3 and 60%, respectively).

In the correspondence analysis (Fig. 7B), siliceous sponges were related to cytotoxic, antimitotic and antibacterial activities, while horny sponges were related to antiviral activity. Antifungal activity was associated with

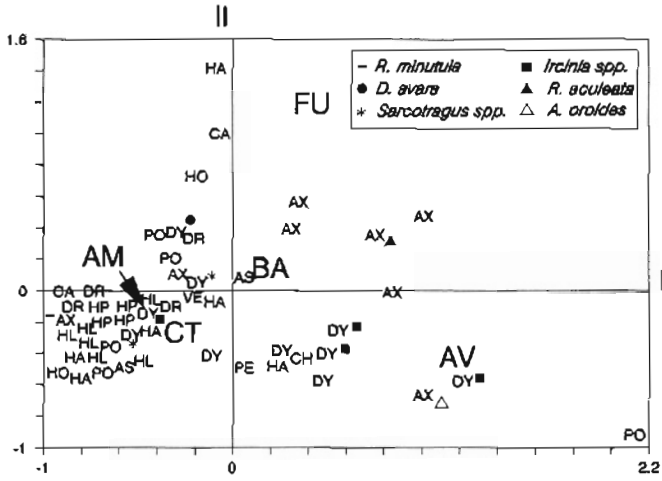


Fig. 5. Distribution of the systematic orders in the first two axes of the correspondence analysis. AS: Astrophorida; HO: Homosclerophorida; CH: Chondrosiida; HA: Hadromerida; AX: Axinellida; PO: Poecilosclerida; HL: Halichondrida; HP: Haplosclerida; PE: Petrosida; DY: Dictyoceratida; VE: Verongida; DR: Dendroceratida; CA: Calcinea. See Table 1 for full genus names of listed species. Abbreviations of bioactivities as in Fig. 3

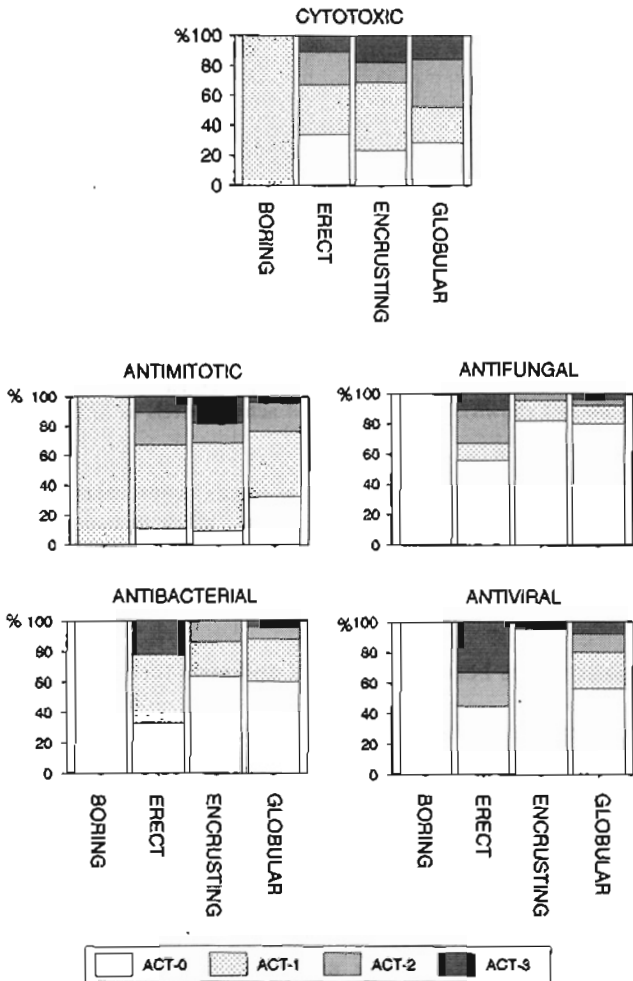


Fig. 6. Percentages of active species displaying the various growth forms considered, for each type of activity tested. Abbreviations of activity levels as in Fig. 2

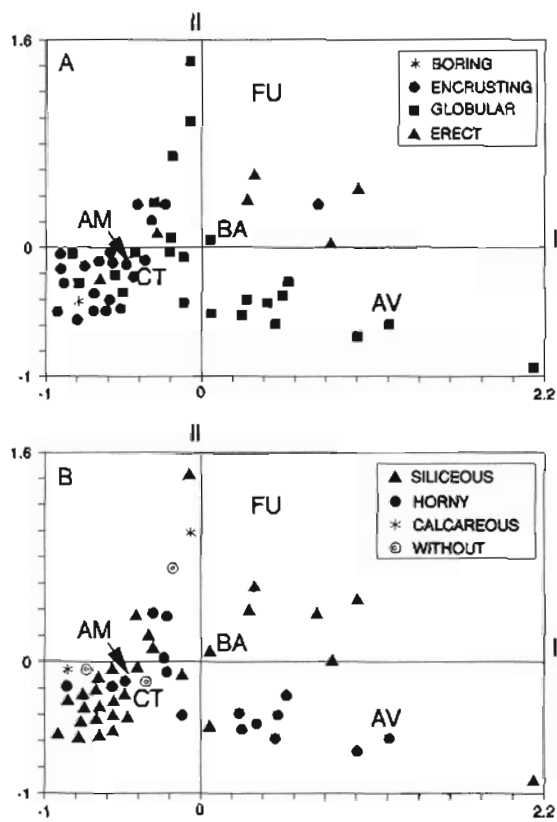


Fig. 7. (A) Distribution of the various growth forms in the factorial plane generated by the first two components of the correspondence analysis. (B) Distribution of the various skeletal types. Abbreviations of bioactivities as in Fig. 3

species possessing siliceous and calcareous skeletons and with those lacking skeletons.

Activities and presence of epibionts

Antibacterial, antifungal and antiviral activities were dependent on the presence/absence of epibionts (significance: $p < 0.05$, $p < 0.05$ and $p < 0.025$, respectively), while the remaining activities were distributed independently (Fig. 9).

Curiously enough, the cases in which the epibiosis was habitual (index 2) corresponded to species exhibiting high indices of activity, especially antimittotic, antifungal and antibacterial activities. However, higher percentages of active species were found among the non-fouled forms than among those occasionally fouled (index 1) (Fig. 9).

In the correspondence analysis (Fig. 10), the species only occasionally supporting epibionts (seasonal forms of hydroids and seaweeds) were mainly associated with cytotoxic and antimittotic activities. Three species with species-specific epibionts were positioned close to the zone of antibacterial activity.

Activities and presence of microsymbionts

Although the percentage of species displaying antimittotic, cytotoxic and, to a lesser extent, antibacterial activ-

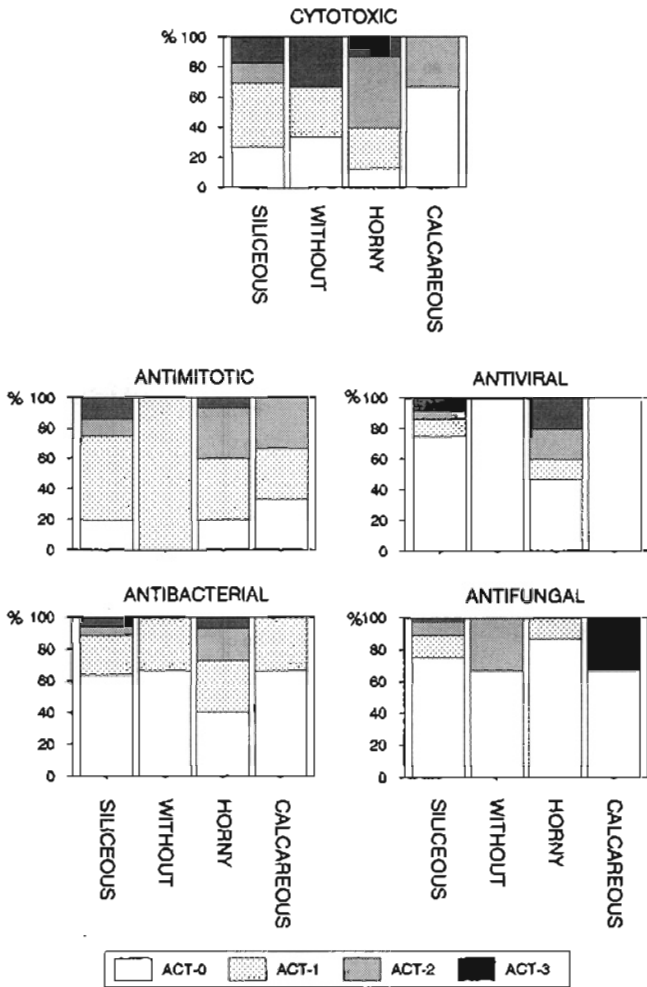


Fig. 8. Percentages of active species possessing the skeletal types considered, for each type of activity tested (abbreviations of activity levels as in Fig. 2)

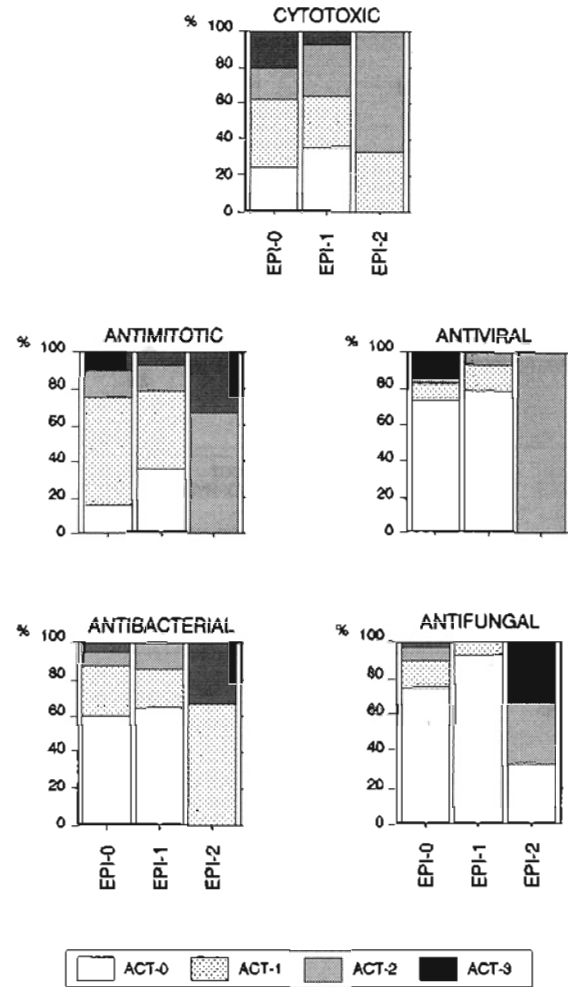


Fig. 9. Percentages of bioactive species displaying various degrees of epibiosis, for each type of activity tested (EPI-0: never fouled; EPI-1: occasionally fouled; EPI-2: habitually fouled). Abbreviations of activity levels as in Fig. 2

ities seemed to be higher among the forms harbouring cyanophyceae, this percentage was significantly dependent on the presence of the algae ($p < 0.05$) only in the case of cytotoxic species. Antifungal and antiviral activities were better represented in species without cyanophyceae (Fig. 11).

The same trends seemed to occur among sponges containing bacteria but, in this case, the information was incomplete (the presence or absence of bacteria is still unknown for some of the species studied; Table 1), and so the interpretations become merely speculative.

In the correspondence analysis, the species containing cyanophyceae were spatially associated with antimittotic, cytotoxic and antibacterial activities (Fig. 10b).

Discussion and conclusions

The production of bioactive substances is a widely distributed characteristic of Mediterranean littoral sponges, as only six of 59 species showed no activity. Although the percentage of sponge species exhibiting antifungal activ-

ity was low relative to that for the remaining activities, it can be considered relatively high compared with other benthic invertebrates (Uriz et al. 1991).

Chemical bioactivity is in most cases a result of the production of secondary metabolites, often common to a cluster of related species (Cimino et al. 1975, Bergquist and Bedford 1978, Bergquist and Wells 1983, Bergquist et al. 1984, Lee and Gilchrist 1985). In this way, similar activity against a specific group of microorganisms and cells may indicate the presence of similar metabolites and, consequently, of systematic affinities among the species. This is the case for *Agelas oroides*, whose position in the correspondence analysis confirms its proximity to the Axinellida (Bergquist 1979) and distance from the Poecilosclerida (Lévi 1973). This reflects the chemical similarity of their metabolites (linear furanoterpenes) to those present in some Axinellida (Cimino et al. 1975) and corroborates results of biochemical studies (Sharma and Burkholder 1967).

The position of *Dysidea avara* near *Pleraplysilla spinifera* (order Dendroceratida) is consistent with the recent proposal (Vacelet et al. 1989) that the family

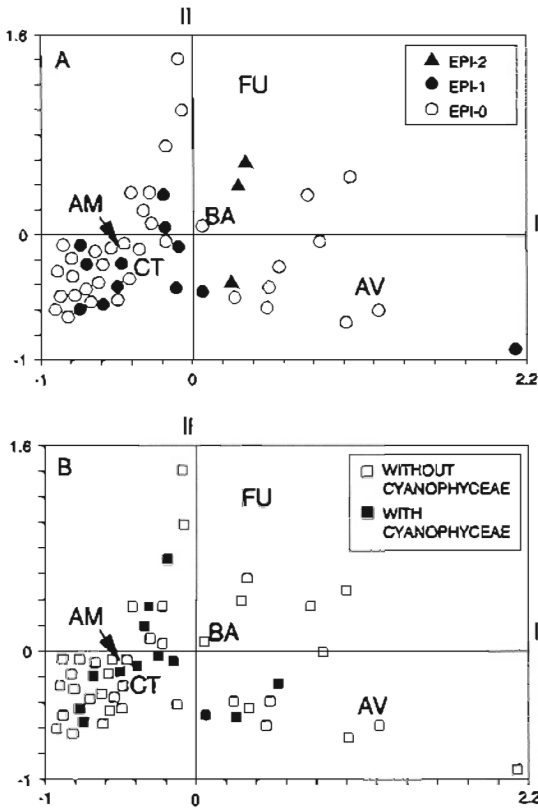


Fig. 10. (A) Distribution of the three indices of epibiosis (see Fig. 9) in the correspondence analysis. (B) Distribution of scores for presence/absence of cyanophyceae in the correspondence analysis. Abbreviations of bioactivities as in Fig. 3

Dysideidae be transferred from the order Dictyoceratida to the Dendroceratida on the basis of histological features. Similarly, the location of *Raspaciona aculeata* indicates more bioactive affinities of this species with the order Axinellida (Lévi 1973, Uriz 1983) than with the Poecilosclerida (Sarà and Siribelli 1960, 1962).

Finally, closer affinities of *Rhabdermia minutula* with Hadromerida (Bergquist 1968) or with Poecilosclerida than with Axinellida could be suspected from this species' position in the analysis. Its affinity with Poecilosclerida would be also supported by the presence in this species of *sigmata* as microscleres.

The separation of species of the genus *Ircinia sensu stricto* from those of the subgenus *Sarcotragus* could reflect the presence of different secondary metabolites, supporting Bergquist's opinion (1980) [followed by Boury-Esnault and Lopes (1985)] that the skeletal differences between these two groups of species are important enough to allow their consideration as two separated genera instead of subgenera.

A negative correlation between the presence of active metabolites and spicules in sponges (Faulkner and Ghiselin 1983) or between the amount of active metabolites and sclerites in gorgonians (Harwell and Fenical 1989) was not evident for Mediterranean littoral sponges. Most species with siliceous and calcareous skeletons, seemingly the most useful anti-predation defences, displayed strong cytotoxic and antimittotic activities, as did species without

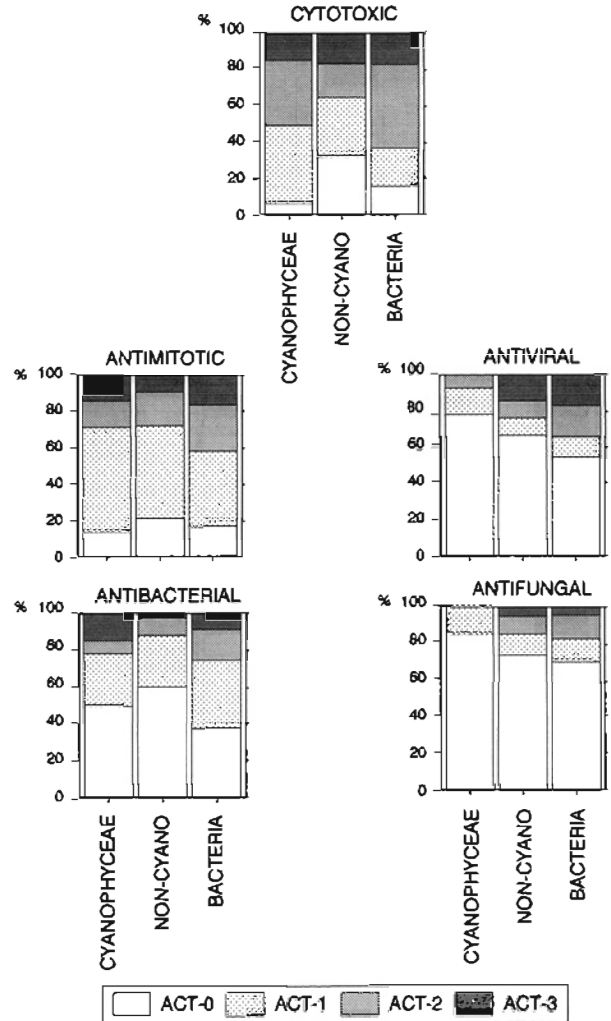


Fig. 11. Percentages of bioactive species displaying various conditions of microsymbiosis, for each type of activity tested. Abbreviations of activity levels as in Fig. 2

skeletons. It has been proven that bioactive metabolites can act as feeding deterrents (e.g. Bakus et al. 1985, Hay and Fenical 1988), but other characteristics besides bioactivity may also play an anti-predation role. Low energy contents (McClintock 1987), the production of unpleasant but non-toxic substances (Green 1977) and the presence of large amounts of microfibrils of collagen in some species could discourage potential predators as do toxic substances. Antibacterial and antiviral activities were well represented in species lacking spicules but containing spongin fibers. However, horny sponges are typically microsymbiont-bearers, and it is not possible to distinguish their respective contribution to the production of active substances.

It is well known that sponges frequently lack epibionts (Bergquist 1979, Uriz et al. 1991). Taking into account that they also are one of the most chemically active of the invertebrate phyla, some correlation between these two parameters could be expected. All the activities examined could play some antifouling role, since they were better represented in non-fouled than in occasionally fouled

species. Nevertheless, only antibacterial, antifungal and antiviral activities were significantly dependent on the presence/absence of epibionts. Consequently, antimicrobial activities appear to be more efficient as antifouling agents than are antimitotic/cytotoxic activities, confirming previous studies (Bakus et al. 1986, Uriz et al. 1991). Nonetheless, antifouling mechanisms involving active substances do not seem potent enough against opportunistic foulers, if one considers that many active species are fouled by seasonal organisms.

Rare instances of chronic epibiosis occurred in species exhibiting a high level of antimicrobial activity (*Axinella verrucosa*, *Axinella damicornis* and *Cacospongia scalaris*). These cases correspond to specific associations (epibiont/basibiont) and suggest evolutionary processes in which chemical defensive strategies developed by the basibiont have induced resistance adaptations in the epibiont, as has been described for predator-prey association (Paul et al. 1990).

The possible association between the absence of epibionts and the presence of chemically mediated bioactivity must be interpreted cautiously. Even though these substances may play an antifouling role in nature, the way in which they are released, their period of activity and the causes of their variability are largely unknown (Bakus et al. 1986). Moreover, other anti-fouling mechanisms exist (Barthel and Wolfrath 1989) that could mask the action of the chemical substances. Non-chemical mechanisms may be common, as indicated by the high percentage of non-fouled sponges which were not chemically active (Fig. 9).

Cytotoxic activity is to some degree related to the presence of cyanophyceae in the studied sponges. These microorganisms may directly produce the active metabolites (Moore 1977), or chemicals may result from metabolic processes associated with symbiotic interactions. Nevertheless, it is not possible, without experimental manipulation, to distinguish the chemical activity due to the cyanophyceae from that due to the sponge. Moreover, cyanophyceae coexist with bacteria in many sponge species (Vacelet and Donadey 1977), causing additional difficulties.

A dual function seems to be displayed by active metabolites of sponges. On the one hand, antimitotic and cytotoxic activities might play a role in competition for substratum, as they predominate in encrusting species (highly dependent on the amount of substratum occupied and occurring in direct contact with neighbours). On the other hand, antifungal, antiviral and antibacterial activities were better represented in erect forms. These erect sponges have avoided substratum competition by growing upwards but, as surface-area-dependent filter feeders, need to maintain their surface free of fouling organisms. Their antimicrobial activities would be related to the first steps of the colonization processes, perhaps inhibiting bacterial, fungal or diatomaceous films (Shim and Jurng 1987, Mellouki et al. 1989). Nevertheless, the biological significance of the antiviral and antifungal activities will to a great extent remain unknown until more information on the role of viruses and fungi in marine ecosystems is obtained.

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