



HAL
open science

SCREENING AND BIOASSAYS FOR TOXIC SUBSTANCES IN SPONGES FROM WESTERN MEDITERRANEAN SEA AND NORTH BRITTANY

G van de Vyver, J Huysecom, J C Braekman, D Daloze

► **To cite this version:**

G van de Vyver, J Huysecom, J C Braekman, D Daloze. SCREENING AND BIOASSAYS FOR TOXIC SUBSTANCES IN SPONGES FROM WESTERN MEDITERRANEAN SEA AND NORTH BRITTANY. *Vie et Milieu / Life & Environment*, 1990, pp.285-291. <hal-03036340>

HAL Id: hal-03036340

<https://hal.science/hal-03036340v1>

Submitted on 2 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

SCREENING AND BIOASSAYS FOR TOXIC SUBSTANCES IN SPONGES FROM WESTERN MEDITERRANEAN SEA AND NORTH BRITTANY

G. VAN DE VYVER⁽¹⁾, J. HUYSECOM⁽¹⁾,
J.C. BRAEKMAN⁽²⁾ and D. DALOZE⁽²⁾

⁽¹⁾ Laboratoire de Biologie Animale et Cellulaire

⁽²⁾ Laboratoire de Chimie Bio-organique, Faculté des Sciences, Université Libre de Bruxelles,
50 avenue F.D. Roosevelt, 1050 Brussels, Belgium

ÉPONGES
TOXICITÉ
ESSAIS BIOLOGIQUES

RÉSUMÉ – La toxicité d'extraits organiques de 27 espèces d'Eponges communes le long des côtes nord de Bretagne et de 26 espèces communes en Méditerranée occidentale a été mise en évidence au moyen de deux tests biologiques. L'un d'eux est un test de cytotoxicité qui évalue, dans des conditions standardisées, la capacité des cellules d'une Eponge d'eau douce dissociée à s'agréger et à reconstituer des Eponges fonctionnelles. L'autre est un test classique d'ichthyotoxicité utilisant le Guppy *Lebistes reticulatus*. Pour les Eponges récoltées le long des côtes de Bretagne, 60 % des espèces manifestent une forme de toxicité, soit dans l'un des deux tests, soit dans les deux, tandis que pour les Eponges méditerranéennes, 65 % des espèces sont apparues toxiques. Nos résultats suggèrent que les Eponges marines produisent des substances toxiques à large spectre d'activité de défense.

SPONGES
TOXICITY
BIOASSAYS

ABSTRACT – Extracts of twenty-seven common sponge species from North Brittany and twenty-six from the Western Mediterranean coast have been investigated for toxicity using two bioassays. One is a new and practical toxicity test which evaluates, under standard conditions, the influence of the extract on the capacity of dissociated sponge cells to aggregate and to reconstitute functional sponges. The other is a classical ichthyotoxicity test using the fish *Lebistes reticulatus*. Sixty percent of the North Brittany sponges studied were found to be active in one or both tests, whereas for the Mediterranean sponges, 65 % of the species appeared to be toxic. Our results suggest that the observed toxicity is not aimed at a particular organism but could reflect a more general biocidal activity required for an overall defense.

INTRODUCTION

Among marine organisms, sponges appear to be one of the phyla richest in toxicogenic species. Indeed, many sponge extracts have been reported to be toxic to a wide range of organisms including microorganisms, invertebrates, vertebrates and algae (Jackson and Buss, 1975; Green, 1977; Bergquist and Bedford, 1978; Bakus, 1981; Amade *et al.*, 1982; Thompson *et al.*, 1985). It has been repeatedly suggested that this toxicity protects sponges against their potential predators and competitors.

In a few cases it has been demonstrated that this toxicity is associated with the presence of specific secondary metabolites (Faulkner, 1984). Several ichthyotoxic metabolites have been isolated from sponges obviously avoided by fishes (Albericci *et al.*, 1982; Groweiss *et al.*, 1983; Braekman and Daloze, 1986). A guanidinoterpene is responsible for the inhibition of coral growth around the base of the oscular chimney of the burrowing sponge *Siphonodictyon* sp. (Sullivan *et al.*, 1983). Antibacterial metabolites, which may be released into seawater, have been shown to display a broad spectrum of biological activities against adults and dispersive stages of algae, invertebrates

and fish (Thompson *et al.*, 1985; Thompson, 1985; Walker *et al.*, 1985).

These data, although still scarce and sometimes based on indirect observations, support the view that the ecological role of sponge secondary metabolites is to minimize predation and/or to serve as weapons in spatial competition.

Recently, we started a research program to study in more detail the role and the mode of action of sponge-produced toxins in the interspecies interactions faced by sponges living in coastal temperate waters. To this end, we needed to develop a practical, reliable and sensitive bioassay to screen for cytotoxicity. This test is described in the present paper as well as its application to the screening of twenty-six sponge species from the Western Mediterranean sea and twenty-seven species from North Brittany. The results are compared with those obtained from a classical ichthyotoxicity test.

MATERIAL AND METHODS

Sponges

Sponges were collected between 0 and 40 m deep by SCUBA diving or by dredging in the Banyuls area (42° 29' N, Western Mediterranean sea, France) and in the intertidal zone or by dredging in the Roscoff area (48° 43' N, North Brittany, Channel, France).

The specimens, cut into small pieces, were placed in methanol for extraction. Corresponding samples were preserved in 70 % ethanol and utilized for species identification. The systematic works of Borgevie *et al.* (1968), Boury-Esnault (1971), Cabioch (1968) and Topsent (1890 to 1945, review in Boury-Esnault 1971) were used to identify the species tested in the present work.

A systematic list of species investigated together with data on their biological characteristics and geographical origin is given in table 1.

Extraction procedure

The methanolic extracts of freshly collected sponges were filtered and the resulting filtrates were concentrated by evaporation under reduced pressure. The residual aqueous solution was then extracted successively with dichloromethane (extract A) and dichloromethane/ethanol 3:2 (extract B). Evaporation to dryness of the remaining aqueous solution yielded extract C containing mostly inorganic salts.

Bioassays

Each sponge extract was tested for toxicity against the fish *Lebistes reticulatus* and the freshwater sponge *Ephydiatia fluviatilis*.

Ichthyotoxicity test

Lebistes reticulatus has been used successfully in previous toxicity studies (Albericci *et al.*, 1982). For each test, two fishes were put into a vial containing 100 ml tapwater added with 5 mg of crude extract dissolved in 1 ml of ethanol. Survival of the fishes was checked after 12 and 24 h. Extracts killing the fish within 12 h are referred to as highly toxic (++), extracts killing the fish after 24 h were referred to as toxic (+).

Cytotoxicity test

This test evaluates, under standard conditions, the sponge extract activity on the viability of dissociated sponge cells and particularly on their capacity to reaggregate and to reconstitute functional sponges. The freshwater sponge *Ephydiatia fluviatilis*, strain δ (Van de Vyver, 1970, 1975), was grown from gemmules in Petri dishes containing mineral medium (Rasmont, 1961). After 7 days of incubation, the sponge was mechanically dissociated by pipetting. The spicules and the empty gemmule shells were discarded and the dissociated cells centrifuged (10 min at 1000 RPM) and resuspended in mineral medium at a final concentration of 10^6 cells/ml. For each test 0.1 mg of the extract to be tested was dissolved in ethanol and added to 1 ml of cell suspension placed in a microtest plate.

About 2 h after dissociation, control cell suspensions of *E. fluviatilis* aggregated into small spherules which fused together during the following hours before to settle on the bottom of the walls (fig. 1). 24 h later, the settled aggregates had reconstituted complete sponges, characterized by a functional aquiferous system (fig. 2). In the presence of toxic extracts, this process is more or less disturbed.

The extract were referred to as highly toxic (++++) when they prevent any aggregation, killing the cell suspension within 2 h (fig. 3,4); referred to as moderately toxic (++) when they allow cell aggregation but that the aggregates degenerate before the time of settlement. Extracts were defined as weakly toxic (+) when the aggregates after formation remained healthy but are unable to settle. Non-toxic extracts did not disturb normal cell aggregation or settlement; they were referred to as inactive (-).

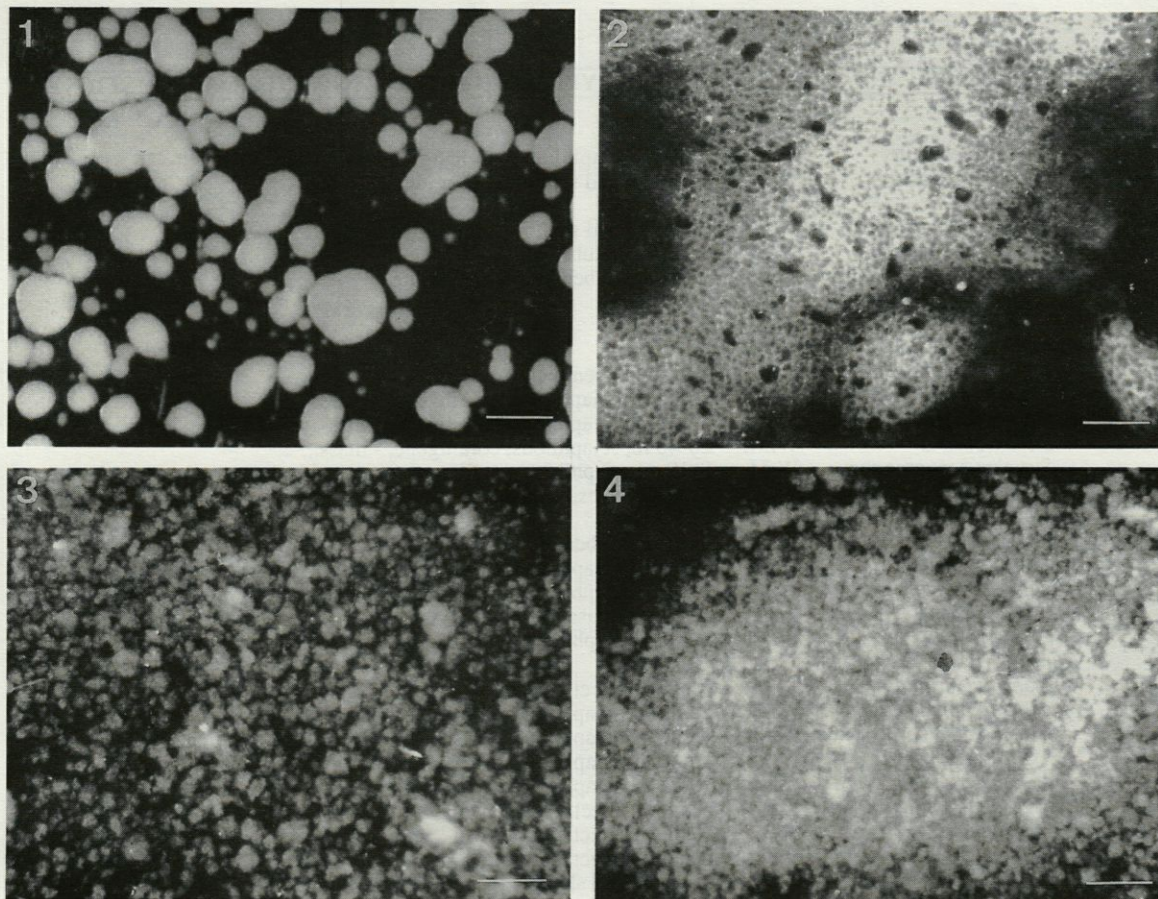


Plate 1. – Control cell suspension of the fresh water sponge *E. fluviatilis*, 2 h after dissociation. Small aggregates are formed. 2, Control cell suspension 24 h later. The settled aggregates reconstitute a functional sponge. 3, Cell suspension of *E. fluviatilis* in presence of 0.1 mg/ml of extracts A + B of *Crambe crambe*, 2 h after dissociation. No aggregation occurred, dead cells are laying on the substrate. 4, The same suspension, 24 h later. Scale bar = 200 μ m.

RESULTS

All the sponges collected have been screened for toxicity by means of two different bioassays, one using a vertebrate organism (ichthyotoxicity test) the other using a lower invertebrate cell suspension (cytotoxicity test). Only results obtained on extracts A and B will be considered, since extract C proved to be always non-toxic.

Western Mediterranean species

The 26 species which were screened for toxicity (table 1, WM) belong to one order of calcareous sponges and six orders of Demosponges. Most examined species were collected in shaded biotopes, on subvertical rocky walls under algae, on overhangs, or on cave ceilings. Two species, *Crambe crambe* and *Hamigera hamigera* very common at low depth, and *Aplysina aerophoba* living between 10-35 m are photophilous, growing on exposed surfaces.

The ichthyotoxic and cytotoxic properties of the species studied are reported in table 2. 14 species (54 %) were found to be toxic to *L. reticulatus*, and among them, 7 were highly toxic. Indeed, extracts A and B of *Topsentia genitrix* and *C. crambe*, extract A of *Acanthella acuta*, *Hemimycala columella* and *Cacospongia scalaris* and extract B of *Tethya aurantium*, *Cliona viridis*, *Dictyonella incisa* and *Reniera fulva* killed the fish within 12 h.

Extracts A and/or B of 4 other species, *Clathrina clathrus*, *Aptos papillata*, *Hamigera hamigera*, *Dysidea tupha* killed the fish between 12 and 24 h, whereas extracts of 12 species (46 %) were totally innocuous at the concentration tested (50 mg/l). 14 species (54 %) showed a cytotoxic activity on the dissociated freshwater sponge cells. For 8 of them, after 2 h of contact with extracts A and B of *A. papillata*, *A. acuta*, *H. columella*, *D. incisa*, *D. tupha* and *C. scalaris*, the cell suspension of *E. fluviatilis* was reduced to a layer of dead cells covering the bottom of the microtest plate. The extracts that killed the dissociated cells

SPECIES	AREA	TYPICAL HABITAT AND CHARACTERISTICS
CALCAREA		
<i>Calcinea</i>		
<i>Clathrina clathrus</i>	W.M.	5-40 m, sciaphilous, overhangs, caves, - encr., yellow
<i>Clathrina coriacea</i>	N.B.	intertidal and shallow, - encr., white
<i>Calcaronea</i>		
<i>Sycon raphanus</i>	N.B.	intertidal and shallow, - tube, greyish white
<i>Grantia compressa</i>	N.B.	intertidal, - tube, white
<i>Leuconia johnstoni</i>	N.B.	intertidal to benthic, - encr., white
DEMOSPONGIAE		
<i>Astrophorida</i>		
<i>Pachymatisma johnstoni</i>	N.B.	shallow to benthic, - mass., grey
<i>Geodia cydonium</i>	W.M.	20-40 m, sciaphilous, coralligene, - mass.,
<i>Stelletta dorsigera</i>	W.M.	15-35 m, sciaphilous, coralligene, - mass.,
<i>Stelletta stelletta</i>	W.M.	20-40 m, sciaphilous, coralligene, - mass.,
<i>Stryphnus mucronatus</i>	W.M.	8-35 m, sciaphilous, coralligene, - mass., black
<i>Chondrosia reniformis</i>	W.M.	5-40 m, caves, - mass., grey to white
<i>Hadromerida</i>		
<i>Tethya aurantium</i>	N.B.	intertidal to benthic, - mass., orange
<i>Tethya aurantium</i>	W.M.	10-35 m, sciaphilous, coralligene, - mass., orange
<i>Aaptos papillata</i>	W.M.	2-40 m, sciaphilous, coralligene, - mass., grey, purple, orange, yellow
<i>Cliona celata</i>	N.B.	benthic, - mass., yellow
<i>Cliona viridis</i>	W.M.	5-40 m, sciaphilous, rocky walls, - mass., green to yellow
<i>Axinella</i>		
<i>Axinella dissimilis</i>	N.B.	benthic, - erect, yellow
<i>Axinella polypoides</i>	W.M.	20-40 m, sciaphilous, bottoms, - erect, yellow
<i>Axinella damicornis</i>	W.M.	15-40 m, sciaphilous, rocky walls, caves, - erect, yellow
<i>Axinella verrucosa</i>	W.M.	15-40 m, sciaphilous, rocky walls, - erect, yellow
<i>Acanthella acuta</i>	W.M.	10-25 m, sciaphilous, rocky walls, - erect, orange
<i>Phakellia ventilabrum</i>	N.B.	benthic, - erect, greyish white
<i>Raspailia hispida</i>	N.B.	benthic, - erect, dark brown
<i>Raspailia ramosa</i>	N.B.	benthic, - erect, dark brown
<i>Polymastia mamillaris</i>	N.B.	intertidal and shallow, - encr. with erect processes, yellow
<i>Polymastia robusta</i>	N.B.	benthic, - mass., yellow
<i>Ficulina ficus</i>	N.B.	benthic, - mass., orange
<i>Halichondria</i>		
<i>Halichondria panicea</i>	N.B.	intertidal and shallow, - encr., green to yellow-orange
<i>Halichondria bowerbanki</i>	N.B.	intertidal and shallow, - encr., green to yellow-orange
<i>Hyeniacidon sanguinea</i>	N.B.	intertidal and shallow, - encr., red
<i>Hemimycale columella</i>	N.B.	shallow to benthic, - encr., pink
<i>Hemimycale columella</i>	W.M.	5-40 m, sciaphilous, rocky walls, - encr., pink
<i>Dictyonella incisa</i>	W.M.	20-40 m, sciaphilous, rocky walls, - mass., orange
<i>Reniera fulva</i>	W.M.	sciaphilous, coralligene, - erect, purple
<i>Petrosia ficiformis</i>	W.M.	5-25 m, sciaphilous, overhangs, caves, - mass., purple to white
<i>Topsentia genitrix</i>	W.M.	35 m, sciaphilous, rocky walls, - mass., yellow
<i>Poecilosclerida</i>		
<i>Myxilla rosacea</i>	N.B.	shallow to benthic, - encr., yellow-orange
<i>Anchinoe fictitius</i>	N.B.	shallow to benthic, - encr., red
<i>Pronax plumosum</i>	N.B.	intertidal to benthic, - encr., red
<i>Ophlitaspongia seriata</i>	N.B.	intertidal to benthic, - encr., red
<i>Crella elegans</i>	W.M.	5-40 m, sciaphilous, rocky walls, - encr. or mass., white
<i>Crambe crambe</i>	W.M.	0-20 m, photophilous, rocky walls, - encr., red
<i>Hamigera hamigera</i>	W.M.	0-10 m, photophilous, rocky walls, - encr., pink
<i>Haplosclerida</i>		
<i>Haliclona elegans</i>	N.B.	intertidal to benthic, - erect, greyish
<i>Haliclona indistincta</i>	N.B.	intertidal to shallow, - encr., greyish
<i>Haliclona viscosa</i>	N.B.	benthic, - mass., light brown
<i>Adocia simulans</i>	N.B.	intertidal to benthic, - erect, greyish
<i>Dictyoceratida</i>		
<i>Dysidea fragilis</i>	N.B.	shallow to benthic, - mass., greyish-white
<i>Dysidea tupha</i>	W.M.	20-35 m, sciaphilous, coralligene, - erect, pink-brown
<i>Cacospongia scalaris</i>	W.M.	
<i>Aplysina aerophoba</i>	W.M.	10-35 m, photophilous, rocky walls, - mass., yellow
<i>Aplysina cavernicola</i>	W.M.	1-12 m, sciaphilous, cave, - mass., yellow
<i>Ircinia fasciculata</i>	W.M.	10-25 m, sciaphilous, rocky walls, - mass., black

Table I. - Systematic list of investigated species, with data on their characteristics and their geographical origin. The growth forms of sponges are divided in 4 groups: encrustations (encr.), massive forms (mass.), erect forms (erect) and tube or vase shapes (tube). W.M. = Western Mediterranean sea, N.B. = North Brittany (Channel).

Sponge species	% of dry weight of sponges		Bioassay results			
	A	B	<i>L. reticulatus</i>		<i>E. fluviatilis</i>	
			A	B	A	B
<i>Clathrina clathrus</i>	1.4	1.1	+	-	+	-
<i>Geodia cydonium</i>	0.6	0.2	-	-	-	-
<i>Chondrosia reniformis</i>	0.3	0.2	-	-	++	-
<i>Stelletta dorsigera</i>	2.15	0.75	-	-	+	-
<i>Stelletta stelletta</i>	0.3	0.3	-	-	-	-
<i>Strychnus mucronatus</i>	2.1	2.3	-	-	-	-
<i>Cliona viridis</i>	1.9	0.6	-	++	-	-
<i>Aaptos papillata</i>	0.2	0.7	+	+	+++	-
<i>Tethya aurantium</i>	0.4	0.15	-	++	-	-
<i>Axinella polypoides</i>	1.2	0.2	-	-	-	-
<i>Axinella damicornis</i>	1.4	1.3	-	-	-	-
<i>Axinella verrucosa</i>	1.9	4.2	-	-	-	++
<i>Acanthella acuta</i>	2.2	0.6	++	+	+++	++
<i>Hemimycale columella</i>	7.0	5.0	++	+	+++	-
<i>Dicyonella incisa</i>	2.2	8.9	-	++	+++	++
<i>Reniera fulva</i>	3.5	2.2	-	++	-	-
<i>Petrosia ficiformis</i>	1.0	0.4	-	-	-	-
<i>Topsentia genitrix</i>	0.45	2.4	++	++	+++	+++
<i>Crella elegans</i>	2.4	1.5	-	-	-	-
<i>Crambe crambe</i>	-	-	++	++	+++	+++
<i>Hamigera hamigera</i>	2.1	0.6	+	-	+	++
<i>Dysidea nupha</i>	2.4	0.05	+	+	+++	++
<i>Aplysina aerophoba</i>	2.1	0.3	-	+	-	+
<i>Aplysina cavernicola</i>	3.3	2.5	-	-	-	-
<i>Ircinia fasciculata</i>	2.9	0.1	-	-	-	-
<i>Cacospongia scalaris</i>	0.4	0.5	++	-	+++	-
<i>Clathrina coriacea</i>	1.8	0.4	+	-	-	-
<i>Sycon ciliatum</i>	3.7	0.2	-	-	-	+++
<i>Grantia compressa</i>	1.6	1.2	+	-	+++	-
<i>Leuconia johnstoni</i>	0.7	0.7	+	-	+++	-
<i>Pachymatisma johnstoni</i>	1.5	0.4	-	++	+++	+++
<i>Tethya aurantium</i>	2.8	0.6	-	-	-	-
<i>Cliona celata</i>	1.2	0.3	-	++	-	+++
<i>Polymastia mamillaris</i>	1.6	1.1	+	-	+++	+++
<i>Polymastia robusta</i>	4.1	4.5	-	-	++	++
<i>Ficulina ficus</i>	2.8	0.8	-	-	-	-
<i>Axinella dissimilis</i>	2.7	1.8	-	-	-	-
<i>Phakellia ventilabrum</i>	0.9	0.5	+	+	+++	+++
<i>Raspailia hispida</i>	3.0	0.1	+	-	-	-
<i>Raspailia ramosa</i>	3.3	0.1	-	-	-	-
<i>Halichondria panicea</i>	2.5	2.3	-	-	-	-
<i>Halichondria bowerbanki</i>	n.a.	n.a.	-	-	-	-
<i>Hyeniacidon sanguinea</i>	0.6	0.1	-	-	-	-
<i>Hemimycale columella</i>	3.4	0.4	-	-	-	-
<i>Myxilla rosacea</i>	3.7	1.0	-	+	+	++
<i>Anchinoe fictitius</i>	5.7	2.1	-	+	+++	+++
<i>Pronax plumosum</i>	7.6	2.0	-	-	-	-
<i>Ophlitaspongia seriata</i>	2.2	0.9	++	-	-	-
<i>Haliclona elegans</i>	6.2	1.2	-	-	-	-
<i>Haliclona indistincta</i>	2.6	2.0	-	-	-	-
<i>Haliclona viscosa</i>	3.0	0.4	+	-	++	+++
<i>Adocia simulans</i>	3.5	0.2	-	-	++	-
<i>Dysidea fragilis</i>	2.1	0.3	+	-	++	++

n.a. : not available

Table II. - Bioassay results for toxic metabolites obtained from Western Mediterranean sponges. (Upper extract A (dichloromethane), Extract B (dichloromethane/ethanol). For *L. reticulatus*, toxic activity is defined by death of the fish either within 12 h (++) or between 12 and 24 h (+). For *E. fluviatilis*, toxicity is defined by the degree of alteration of cell aggregation and sponge reconstitution. Extracts are referred to as highly toxic (+++), moderately toxic (++) or weakly toxic (+).

before the early aggregation were considered as highly toxic. The extracts of three species; *Chondrosia reniformis* (extract A), *Axinella verrucosa* and *Hamigera hamigera* (extract B) allowed the constitution of aggregates, but the aggregates were unable to settle and degenerated after a few hours. These extracts were considered as moderately toxic.

Extract A of *C. clathrus* and *Stelletta dorsigera*, and extract B of *A. aerophoba* allowed aggregation to occur normally. But despite the fact that the aggregates looked healthy for several days, they never settled or reconstituted organized sponges. 12 species (46 %) were completely non-toxic to *E. fluviatilis*. They were referred to as inactive (-).

The results obtained from both bioassays were in complete agreement for 20 species among the 26 tested. Indeed, 11 species (42 %) were lethal to the fish and the sponge cells, whereas 9 (35 %) were completely inactive. In contrast, 3 species (*Tethya aurantium*, *Cliona viridis*, *Reniera fulva*) were lethal only to the fish and three others (*C. reniformis*, *S. dorsigera*, *A. verrucosa*) only to *E. fluviatilis*.

North Brittany species

27 species which were investigated for toxic activity (Table 1, N.B.) belong to two orders of Calcarea sponges and seven orders of Demosponges.

Most examined species were non-cryptic and were collected either on pebbly bottoms or on exposed rocky walls. Nevertheless, the four calcarean sponges tested (*C. coriacea*, *S. ciliatum*, *Grantia compressa*, *Leuconia johnstoni*) and the Demosponge *Pachymatisma johnstoni* were collected in shaded biotopes, under algae or on cave ceilings. Similarly, *Polymastia mamillaris*, growing in the open light appears shaded by a layer of sand.

The ichthyotoxicity and cytotoxicity properties of all the species studied are reported in table II.

13 species (48 %) were found to be toxic to *L. reticulatus*. Extract A of *Ophlitaspongia seriata* and extract B of *P. johnstoni* and *Ciona celata* killing the fish within twelve hours were referred to as highly toxic. Extracts A and/or B obtained from the 10 other species *Phakellia ventilabrum*, (extract A and B), *C. coriacea*, *Grantia compressa*, *L. johnstoni*, *P. mamillaris*, *Raspailia hispida*, *Haliclona viscosa*, *Dysidea fragilis* (extract A), *Myxilla rosacea* and *Anchinoe fictitius* (extract B) were lethal to the fish after more than 20 h.

Finally, 14 species (52 %), one calcarean sponge and 13 Demosponges were non-toxic at the concentration tested (50 mg/l).

Extracts of 13 species modified the pattern of aggregation settlement and sponge reconstitution of *E. fluviatilis* cell suspensions.

Extracts A and B of *P. johnstoni*, *P. mamillaris*, *Phakellia ventilabrum*, *Anchinoe fictitus*, extract A of *G. compressa*, *L. johnstoni* and extract B of *S. ciliatum* and *C. celata* that killed the cell suspensions of *E. fluviatilis* within 2 h were considered as highly toxic.

Extracts A and B of *Polymastia robusta*, extract A of *Haliclona indistincta*, *H. viscosa* and *adocia simulans*, extract B of *Myxilla rosacea* and *Dysidea fragilis*, that allowed cell aggregation only, were referred to as moderately toxic. Extract A of *M. rosacea* permitted aggregation to occur normally. The aggregates looked healthy for several days but they never settled.

Both extracts A and B of 14 species, one calcarean and thirteen Demosponges appeared to be non-toxic.

Comparing the results obtained from both bioassays, it appeared that for 21 species among the 17 screened for the North Brittany coast, the results are in complete agreement. 10 species (37 %) appeared to be toxic both to *L. reticulatus* and *E. fluviatilis*, whereas, 11 (41 %) were completely inactive in the two tests. In contrast, 3 species (*C. coriacea*, *Raspailia hispida*, *Ophlitaspongia seriata*) were found to be lethal only to the fish and three others (*S. ciliatum*, *P. robusta*, *Adocia simulans*) were toxic only to the sponge.

DISCUSSION

Twenty-six sponge species collected along the Western Mediterranean sea coast and 27 species from North Brittany have been screened for toxic metabolites by two different bioassays, one using a vertebrate organism (ichthyotoxicity test), the other using an invertebrate cell suspension (cytotoxicity test). The measurement of viability of *L. reticulatus* in presence of sponge extracts allows an easy detection of ichthyotoxic activity. The second screening method is based on the analysis of reactions of isolated cells obtained from mechanical dissociation of the freshwater sponge *E. fluviatilis*. This new biological test allows to work at the cellular level in standardized conditions. It is easy to perform and since it requires few quantities of extracts, it allows to multiply the number of experiments. Repetitive experiments with the same sponge extract demonstrate that the test is qualitatively sensitive and reliable.

For the sponges tested in the present work, it is worth mentioning that few differences have been detected between the results obtained from both bioassays. Indeed, the degree of similarity reaches 60 % for the North Brittany species and 65 % for the Western Mediterranean sea species. These results suggest that the observed toxicity is not aimed at a particular organism but could re-

flect a more general biocidal activity required for an all-directional defense.

It has been demonstrated by Bakus and Green (1974) that the percentage of ichthyotoxic species is decreasing with increasing latitude. The high percentage of toxic species we found along the European coasts was quite unexpected. Indeed, in their screening along the West American coast, at a latitude corresponding to that of North Brittany, Bakus and Green (1974) only found 9 % of ichthyotoxic sponge species, whereas we obtained ichthyotoxic extracts from 48 % of the species tested.

A comparison of our screening results (table II) with the systematic classification provided in table I shows that toxic activity is found in Calcarean sponges and all orders of Demosponges.

For the species studied, no correlation between toxicity and either depth or microhabitat has been observed. The incidence of toxic openly exposed sponge species was not found to be higher than the incidence of toxic cryptic species, e.g. among the three Mediterranean photophilous species, *Crambe crambe* is highly toxic, *Hamigera hamigera* moderately toxic, and *Aplysina aerophoba* non toxic at all. Our observations restricted to temperate zone species differ from those made in coral reefs by Green (1977) and Bakus (1981) who reported that the most common exposed sponges prove to be highly toxic to fish. This discrepancy may be explained by the increased species diversity in tropical shallow water fishes, resulting in a more intense food competition and hence the natural selection of chemically protected species.

No relationship was found either between toxicity and morphological characteristics of sponges like growth form or color. Among the four most ichthyotoxic species, one is grey, another yellow, a third red and the last brown. Similarly, all kinds of color were observed in non-toxic species. Absence of aposematic color in toxic sponges has also been reported for Caribbean species (Bakus and Thun, 1978).

The toxicity tests described in this paper allowed us to select a series of sponge species for chemical examination. This led to the isolation and structure determination of several new toxic metabolites, e.g., sesquiterpene isonitriles from *A. acuta* (Braekman *et al.*, 1987), indole derivatives from *T. genitrix* (Bartik *et al.*, 1987, Braekman *et al.*, 1989) and a polyoxygenated steroid from *D. tupha* (Braekman *et al.*, 1988).

It is suggested that sponge toxic substances could be multipurpose defensive weapons whose broad spectrum of bioactivities may have been selected for by the diversity of predators and competitors they have to face.

ACKNOWLEDGEMENTS : This study was supported by grants from the FRFC (n 2.451585) and

the «Ministère de l'Emploi et du Travail» (CST. 20564). The authors are indebted to Prof. J. Soyer, director of the Arago Laboratoire (Banyuls-sur-Mer, France), and to Prof. P. Lasserre, director of the Roscoff Marine Station (Brittany, France) for provision of laboratory facilities and services. They gratefully acknowledge the competent assistance given by the Laboratoire Arago's professional divers, J. Mabit and G. Boyer. They want to thank Dr. N. Boury-Esnault for helpful discussions on sponge taxonomy.

REFERENCE

- ALBERICCI M., J.C. BRAEKMAN, D. DALOZE, B. TURSCH, 1982. The chemistry of three norsesterterpene peroxides from the sponge *Sigmosceptrella laevis*. *Tetrahedron* **38** : 1881-1890.
- AMADE Ph., D. PESANDO, L. CHEVOLOT, 1982. Antimicrobial activities of marine sponges from French Polynesia and Brittany. *Mar. Biol.* **70** : 223-228.
- BAKUS G.J., 1981. Chemical defense mechanisms on the Great barrier reef, Australia. *Science* **211** : 497-499.
- BAKUS G.J., G. GREEN, 1974. Toxicity in sponges and holothurians: a geographic pattern. *Science* **185** : 951-953.
- BAKUS G.J., M.A. THUN, 1978. Bioassays on the toxicity of Caribbean sponges. In: *Biologie des spongiaires*. Coll. Int. CNRS, 291 : 417-422. Levi C. and Boury-Esnault N. eds.
- BARTIK K., J.C. BRAEKMAN, D. DALOZE, C. STOLLER, J. HUYSECOM, G. VAN DE VYVER, R. OTTINGER, 1987. Topsentins, new toxic bis-indole alkaloids from the marine sponge *Topsentia genitrix*. *Can. J. Chem.* **65** : 2118-2121.
- BERGQUIST P.R., J.J. BEDFORD, 1978. The incidence of antibacterial activity in Marine Demospongiae, systematic and geographic considerations. *Mar. Biol.* **46** : 215-221.
- BOROJEVIC R., L. CABIOCH, C. LEVI, 1968. Inventaire de la faune marine de Roscoff. Spongiaires.
- BOURY-ESNAULT N., 1971. Spongiaires de la zone rocheuse littorale de Banyuls sur mer. I. Ecologie et Répartition. *Vie Milieu* **22** : 159-191. II. Systématique. *Vie Milieu* **22** : 287-350.
- BRAEKMAN J.C., D. DALOZE, F. DENEUBOURG, J. HUYSECOM, G. VAN DE VYVER, 1987. 1-Isocyno-aromadendrane, a new isonitrile sesquiterpene from the sponge *Acanthella acuta*. *Bull. Soc. Chim. Belg.* **96** : 539-543.
- BRAEKMAN J.C., D. DALOZE, B. MOUSSIAUX, G. VAN DE VYVER, R. RICCIO, 1988. Cholest-6-en-11b,19-epoxy-3b,5a,8a,9a-tetrol, a novel polyoxy-generated steroid from the sponge *Dysidea tupa*. *Bull. Soc. Chim. Belg.* **97** : 293-296.
- BRAEKMAN J.C., D. DALOZE, B. MOUSSIAUX, C. STOLLER, F. DENEUBOURG, 1989. Sponge secondary metabolites: new results. *Pure Appl. Chem.* **61** : 509-512.
- CABIOCH L., 1968. Contribution à la connaissance de la faune des spongiaires de la Manche Occidentale. Demosponges de la région de Roscoff. *Cah. Biol. Mar.* **9** : 211-246.
- FAULKNER D.J., 1984. Marine natural products: metabolites of marine invertebrates. *Natural Products Reports*, **1** : 551-598.
- GREEN G., 1977. Ecology of toxicity in marine sponges. *Mar. Biol.* **40** : 207-215.
- GROWEISS A., U. SHMUELI, Y. KASHMAN, 1983. Marine toxins of *Latrunculia magnifica*. *J. Org. Chem.* **48** : 3512-3516.
- JACKSON J.B.C., L.W. BUSS, 1975. Allelopathy and spatial competition among coral reef invertebrates. *Proc. Nat. Acad. Sci. USA* **72** : 5160-5163.
- RASMONT R., 1961. Une technique de culture des éponges d'eau douce en milieu contrôlé. *Ann. Soc. Roy. Zool. Belg.* **91** : 147-156.
- SULLIVAN B., D.J. FAULKNER, L. WEBB, 1983. Siphonodictidine, a metabolite of the burrowing sponge *Siphonodictyon* sp. that inhibits coral growth. *Science* **221** : 1175-1176.
- THOMPSON J.E., R.P. WALKER, D.J. FAULKNER, 1985. Screening and bioassays for biologically-active substances from forty marine sponge species from San Diego, California, USA. *Mar. Biol.* **88** : 11-22.
- THOMPSON J.E., 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. I. Biological evidence. *Mar. Biol.* **88** : 23-26.
- VAN DE VYVER G., 1970. La non-confluence intraspécifique chez les spongiaires et la notion d'individu. *Ann. Embryol. Morph.* **3** : 251-262.
- VAN DE VYVER G., 1975. Phenomena of cellular recognition in sponges. *Curr. Top. Dev. Biol.* **10** : 123-140.
- WALKER R.P., J.E. THOMPSON, D.J. FAULKNER, 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. II. Chemical evidence. *Mar. Biol.* **88** : 27-32.

Reçu le 14 octobre 1988; received October 14, 1988

Accepté le 29 mai 1989; accepted May 29, 1989