COMPARATIVE ICHTHYOTOXICITY OF SHALLOW AND DEEP WATER SPONGES OF NEW CALEDONIA

S.C. LA BARRA, D. LAURENT, P. SAMMARCO, W.T. WILLIAMS and J. COLL

1UR 707, ORSTOM, B.P. A 5, Nouméa, New Caledonia
2Australian Institute of Marine Science, P.M.B. N°3, MSD, Townsville, QLD 4810, Australia
3Department of Chemistry and Biochemistry, James Cook University of North Queensland, Townsville, QLD 4811, Australia

ABSTRACT

We have conducted a survey of toxicity distribution with depth of common marine sponges of the Southern province of New Caledonia. Test fish Gambusia affinis (Vertebrata, Pisces) were exposed to aqueous macerates from 30 shallow water and 30 deep water sponges. Mortality counts and relative toxicities as evidenced through gradual behavioral changes were recorded against a geometric time scale. The responses exhibited by the fish ranged from rapid mortality and varying levels of distress or narcotization to effects undistinguishable from controls. Sponges exhibiting similar behavioral patterns (from 8 computer-defined patterns) were classified into 5 toxicity groups, falling into 3 broad categories: 100% lethal, harmful to toxic, and non-toxic. Eighty percent bear morphological defences species. Mortality rates of the sponges were at least harmful to toxic, deep sponges being generally more lethal. Overall toxicities being comparable, most sponges bear morphological defences as well, though deep sponges rely on different structures than shallow species.

INTRODUCTION

Marine invertebrates are known to contain a wide range of organic molecules, which correspond to secondary metabolites found in terrestrial plants (Penny, 1975; Selove and Crewe, 1980; Littler et al., 1985). Coral reefs have received a lot of attention by organic chemists and marine ecologists because of the high diversity of species assembled as communities, which offers a surprisingly large number of organic molecules of the same types as encountered on land (Brown et al., 1970; Ktridge et al., 1974).

Sponges are among the most common and the best studied group by chemists (Faunlner, 1987). Often independently, ecologists have studied the toxicity of reef sponges and correlated it to fish predation (Randall and Hartman, 1968; Bakus and Green, 1974; Green, 1977) competition for space, fouling by algae, microorganisms and epizoic communities, and even reproductive aspects of individual species (a review is given by Bakus, 1986).

Fedding deterrence and structural adaptations were often found to have selective advantages in non-cryptic species lacking toxic chemicals and yet not subjected to visible levels of predation (Bakus and Thun, 1979; Green, 1981; La Barre et al., 1986; Sammarco et al., 1987). Comparative toxicity studies between different localities, geographical areas and latitudes have highlighted evolutionary aspects of this primitive phylum, by contrasting prevailing environmental conditions (Green, 1977). This paper represents the first chemical ecology work on New Caledonian sponges, and compares material found in the well known shallow lagoon environment with the almost unknown deep subreef slopes. Although we have extensively relied on techniques devised by us on studies of Great Barrier Reef spongiomycetarians, the present work must be regarded as preliminary until we know more about the sponges and their habitat below diving depths.

MATERIALS AND METHODS

Collection of specimens

Thirty shallow water sponge specimens were collected from various locations around New Caledonia (166°22'S) between January 1986 and June 1987, at depths ranging from 10 cm to 35 meters of depth. Specimens utilized in toxicity testing were placed in labelled plastic bags and deep frozen. Corresponding specimens were preserved in 70% ethanol and utilized as reference samples for taxonomic determinations. Identifications were made by Claude Levi of the Museum d'Histoire Naturelle de Paris, and illustrated references compiled by Pierre Laboute of ORSTOM at Nouméa.

Toxicity tests

Ichthyotoxicity tests were adapted from techniques we used in studies of soft coral toxicity in the central region of Great Barrier Reef, Australia (Coll et al., 1982; Coll and Sammarco, 1983; La Barre et al., 1986), themselves inspired from procedures developed by Yamanouchi (1955) on holothurians and Bakus and Thun (1979) on sponges. Aqueous extracts of sponges were prepared by blending 50 grams of frozen tissue with 100 ml of freshwater and centrifuging the macerate at 2,000 rpm for 30 mins. The resulting supernatant afforded two portions (50 ml) for the replicate ichthyotoxicity bioassay utilizing Gambusia affinis (Baer and Girard) as the test organism. The test aquarium consisted of rectangular glass structures subdivided into 6 sets of two replicate compartments; each held a volume of about 3 litres. Divisions between the replicate compartments were translucent to help visually isolate the fish lots from one another.

Five adults or subadult fish (100 to 300 mg in weight and 19-30 mm in length) were placed in each compartment in 100 ml of water. Different sized fish were distributed uniformly among test containers. It was assumed that, in general, sexes among the 830 fish used were randomly distributed among test aquaria.

Observations on the behaviour of fish were made as follows:
- Location (surface, mid-water or bottom);
- Orientation (normal, lateral, dorsal roll, or both lateral and vertical roll);
- Movement (none, hyperactive, normal, hyperactive);
- Fin activity (none, hyperactive, normal, hyperactive);
- Response to visual stimulus: a sudden shading was caused by blocking the clear front of the compartments at the same time by black surfaces.

1,2 q.v. Acknowledgements.

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Cote : 3

Coll : B
Behavioural patterns were recorded for each fish at t₀ prior to the addition of extracts and after addition following a geometric time scale: 22 min. 45 min. 1.5 h. 3 h. 6 h and 12 h. Physical and chemical properties of the freshwater in the test aquaria were not monitored as it was believed that any effective changes in these characteristics would become evident through the behaviour of control fish.

Numerical methods

The original data where then analysed with respect to treatment and controls; six toxicity groups emerged, each group comprising sponge extracts were subjected to multivariate analysis by computer. They yielded a data-set consisting of 120 x 7 (840) "test occasions" (60 coral species and 60 controls) on 7 successive occasions. Each "test occasion", irrespective of treatment, was characterized by 7 observations on 10 fish and each observation was regarded as a multinomial attribute (Williams and Lance, 1977) with a possible maximum of 4 states.

The data set was classified using the procedure described in Coll et al. (1982), modified to suit the specification of the resulting matrix: eight relatively discrete behavioural states were defined, each summarising a series of responses. The original data were then analyzed with respect to treatment and controls; six toxicity groups emerged, each group comprising sponges deemed similar in their relation to their overall toxicity and their passage through the 7 behavioural states with time.

<table>
<thead>
<tr>
<th>Response code</th>
<th>Location</th>
<th>Orientation</th>
<th>Movement</th>
<th>Fin activity</th>
<th>Response to visual stimulation</th>
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<tr>
<td>A</td>
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<td>none</td>
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</tr>
<tr>
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<td>mid</td>
<td>lateral roll</td>
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<td>vertical roll</td>
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<td>D</td>
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<td>both</td>
<td>hyperactive</td>
<td>hyperactive</td>
<td>hyperactive</td>
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**State 1**:  
A 0.349 0.735 9.072 9.494 9.867  
B 0.120 9.108 0.587 0.205 0.096  
C 9.530 0.084 0.036 0.096 0.036  
D 0.000 0.072 0.205 0.205 0.000  

**State 2**:  
A 2.349 8.628 5.488 4.558 5.608  
B 1.116 1.279 3.884 4.047 3.581  
C 6.535 0.070 0.395 0.674 0.603  
D 0.000 0.023 0.233 0.721 0.116  

**State 3**:  
A 3.327 9.959 1.122 0.429 0.286  
B 3.592 0.000 7.857 7.490 1.224  
C 3.082 0.041 0.837 1.816 0.551  
D 0.000 0.000 0.184 0.263 0.939  

**State 4**:  
A 8.626 9.609 2.609 1.348 0.870  
B 0.609 0.174 6.913 4.000 7.957  
C 0.565 0.217 0.435 4.522 1.174  
D 0.000 0.000 0.043 0.130 0.000  

**State 5**:  
A 2.228 9.782 2.652 1.619 1.533  
B 2.642 0.131 3.436 2.750 3.489  
C 5.130 0.076 3.826 5.424 4.489  
D 0.000 0.018 0.065 0.206 0.489  

**State 6**:  
A 0.551 9.092 0.007 0.003 0.003  
B 8.982 0.003 0.098 0.029 0.073  
C 0.467 0.003 9.358 9.408 9.018  
D 0.000 0.000 0.536 0.558 0.905  

**State 7**:  
A 3.294 9.051 0.348 0.255 0.088  
B 2.073 0.039 0.461 0.216 0.225  
C 3.832 0.010 9.098 9.431 8.539  
D 0.000 0.000 0.098 0.098 1.147  

**State 8**:  
A 1.213 10.000 0.006 0.006 0.000  
B 6.253 0.000 0.115 0.011 0.161  
C 2.534 0.000 9.649 9.833 9.690  
D 0.000 0.000 0.230 0.149 0.149  

Table 1. Gambusia affinis. Behavioural states in individuals exposed to crude extracts from various sponges. Entries represent proportion of fish exhibiting a particular behaviour. States determined from the collection of extensive behavioural data and analysed by multivariate computer techniques (see text for methods).
The sponges tested exhibited a wide range of toxicity (Table 2). The responses exhibited by the test fish were grouped by multivariate analysis into 8 distinct behavioural states (Table 1).

### RESULTS

The sponges tested exhibited a wide range of toxicity (Table 2). The responses exhibited by the test fish were grouped by multivariate analysis into 8 distinct behavioural states (Table 1).
States 2 and 3 described sublethal to lethal conditions, with alternation of deep sleepiness with no fin movement and sudden spurts of activity from bottom to surface, with rapid fin and gill movements wherever observable, followed by sinking with loss of orientation (lateral roll, non-rolling both). Extracts with prolonged effects caused some paralysis in pectoral fin movements, the fish entire bodies swaying from side to side from tail motion. The proportion of hyporeactive and hyporesponsive fish is dramatically increased between state 3 and state 2, mostly because of the much higher number of dead fish in the latter.

States 4 and 5 characterized more or less severe changes in behavior without loss of orientation during swimming, i.e. depressive stages with fish usually gathered at the bottom of the tank, within tactile range of one another, sometimes followed by periods of activity and hyperreactivity to sudden light changes. Defecation often important and irregular fin movements during swimming stages were regarded as signs of stress. Fish usually recovered from intoxication after the experiment, if placed in normal conditions.

States 6, 7 and 8 reflect normal behavior: in state 8 fish are not mobile but their orienta-

### TOXICITY CATEGORIES AND GROUPS

<table>
<thead>
<tr>
<th>GENERA REPRESENTED</th>
<th>100 % lethal</th>
<th>harmful to toxic</th>
<th>non toxic</th>
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<td>II</td>
<td>III</td>
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<td>* Podocampa</td>
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<td>* Xenospongia</td>
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<td>* Keida</td>
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<td>* Loxaliana</td>
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<td>* Hymenooidea</td>
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<td>* Axinellida</td>
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<td>* Phoronema</td>
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<td>* Tethya</td>
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<td>Gollinopria</td>
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<td>* Hedrilla</td>
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<td>* Echinochallina</td>
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<td>* Spinogallia</td>
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<td>* Appendogallia</td>
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<td>* Chondrogesis</td>
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Table 3. Summary of degree of toxicity by sponge genus and variability of toxicity within a genus. Table body: number of specimens falling into a particular toxicity group. Asterisk represent deep group *. 
tion and responses are perfectly normal, and in states 7 and 6 fish are swimming slowly or normally. These three states are all represented at t, before addition of the extracts into the tank, and can be regarded as the results of normal interactions between fish and between fish and their environment.

Based on similarity of sequences through the above states with time, and on death scores, individual sponge species were sorted into 6 toxicity groups. (Table 2). The first two groups yielded 100% mortality of the test organisms, with the first group inducing death within the first 22 minutes of the experiment; the second group was also 100% lethal, but encompassed rapid intoxications (all dead at 90 min) and delayed actions (all dead at up to 12 hr). A wide range of genera (Table 3) were represented in the 100% lethal groups, two thirds of which were from deep bottom trawlings: Ircinia, Philodictyon (2 species), Podospongia, Actinospongia Reidia (2 species), Neosiphonia, Phoronema, Thezya, plus two new and yet undetermined genera. Various pharmacological tests on raw and purified fractions of these specimens have confirmed highly potent and sometimes quite selective toxicities (in progress). Except for Phoronema, these genera were confined to groups I and II. Shallow genera included Halichon, an unknown Calcarea, Toxochalinia, Domitria, Asyni- sa and a member of the Myxillidae family.

Groups III and IV clearly induced severely abnormal behaviour in the test organisms, with resulting death of 10% to 90% of the fish in group III. Survivors of groups III and IV were not capable of recovering from intoxication after being placed in normal post-experiment conditions. A wide range of genera were also represented here, deep specimens included the genera Patroia, Corallistes (2 species), Cladorhiza, Steleia (2 species) and Geodia (3 species). Shallow specimens included Haliotons (2 species), Callyon- gia, Dendryll (2 species), Myxella, Cliona (3 spe- cies), Hateronema, Spirastrella, Liecing, Echinochel- ling and two undetermined entities.

Group V and IV encompassed specimens presenting low or transient toxicities at worst from which test organisms usually recovered after the experiment. Six deep water genera figured here, four of which confined to these two groups: Jasple, Regadrella and two undetermined genera. Non toxic shallow genera included Siphonochalinia, Clathria, Spionella, Chondrosia and axinellid and one undetermined sponge, plus Phoronema and Cliona, also represented in toxic groups.

All controls were confined to group IV except one multiple control classified in group V.

Altogether, 80% of the samples caused abnormal behaviour in Cambusia affinis and the remaining sponges (plus all controls) showed no observable effect or at worst mild and transient behavioural changes on some but not all fish. 5% of the sponges induced almost instantaneous death to all fish and 25% of the sponges killed all fish with some delay: this "100% lethal" category encompasses toxicity groups I and II. The "harmful to toxic" category represents half of the specimens tested (groups III and IV) and the "non toxic" category only 20% (groups V and IV). These results are presented in Figure 1 as histograms.

DISCUSSION

In this study, we have determined the relative toxicities of 60 specimens of common sponges (30 shallow-dwelling and 30 deep subreefal) representing 42 genera, and 57 distinct species. A wide range of responses was observed in test organisms, in accordance to similar studies on sponge toxicities (Bakus and Thur, 1979 and a review by Bakus, 1976). Irrespective of final toxicity scores, we observed effects ranging from almost immediate death to barely observable discomfort, some extracts eliciting rapidly stabilised symptoms, others producing gradual or fluctuating effects. This variability was reflected qualitatively. Some examples showed more or less intense narcotisation were alertness, metabolism and posture maintenance where decreasing to least energy-requiring states. Other examples showed alternations of hyperactive and sedate phases, along with numerous individual variations (e.g. strong defecations, body contacts, strong hyper-reactivity to sudden light changes, bith- giving by gravid females, leaps above surface (etc.), all indicative of stress with possible subsequent recovery.

A systematic evaluation of the chemical composition of these extracts would be necessary to determine if the overall toxicities can be attributed to varying concentrations of few selected molecules, independently of subtle variations in "mild" behavioural states. Synergistic action (positive or negative) of pairs or groups of molecules may be necessary to activate certain biological receptors and the added effects of confinement for several hours and sponge toxins may exceed expected levels, as suggested in studies of soft coral toxins (Coll et al., 1982).

However, the vast array of biological activities exhibited by the sponges in pharmacological tests currently underway in our laboratories will allow the species-specificity of the behavioural sequences in this study (pers. obs.), implying that more than just a few receptors and metabolic processes
are affected in the test fish. It is known from the rapidly growing marine natural substances literature (reviewed by Faulkner, 1985 and 1987) that in sponges, organic compounds are extremely varied and encompass several classes of metabolites. Furthermore, the identification of chemical components of raw extracts may provide species-specific fingerprints of value to sponge taxonomists, as in gorgonian (Gerhart, 1983). The concentration of a single toxic metabolite may vary several orders of magnitude between closely related congeners, species, or fluctuate seasonally in a given intra-specific population (Alan Ahond, pers. comm.) but the same metabolite is rarely found across unrelated genera. We indeed found that 37 of the genera tested (18%) were represented in one category of toxicity only, which provides further evidence of the chemical diversity responsible for our bioassay results.

The other interesting findings relate to habitat. We assumed that we dealt in both situations (shallow and deep) with commonly encountered species. In both cases the many genera and species were equally represented (29 shallow species for 21 genera; 28 deep species for 21 genera and one mixed genus). Few specimens appeared to be conspecific polymorphs; they yielded comparable toxicity scores and behavioural sequences (Phaedonicyton 25 and 95; Halisigia 47 and 33; Candidia 43 and 45). In general, 100% lethal sponges were found in deep trawlings (63% of the species, i.e. 2/3) but approximately 2/3 of equally distributed between the two biota. With such scores, it is not possible to decide whether commonly encountered species are more toxic in sunlit coral reefs or dark substrates. As for shallow sponges, highly toxic individuals tended to lack effective structural armourment, whereas mildly or non toxic species tended to armoured with calcareous shells, needle sharp sclerites (deep) or tough, leathery tissue (shallow). However, very little is known of predation and other selective pressures at such depths, and the correlation between the occurrence of one type of defense and the absence of the other is weak.

Comparative studies of sponge toxicities by Baku (1964, 1974) and Green (1977) have included species from tropical and temperate localities, but no bathymetric correlations have been recorded so far. These authors postulated that predation by reef fish was a strongly influencing factor in favouring diversity in chemical defenses during evolutionary times, but Randall and Hartman (1968) argue that sponges feeders are modern teleosts, and that the long existing and widespread toxicity of sponges is the result of other selective pressures as well. Dayton (1974) found that high biomass and low species diversity of antarctic sponge populations are attributed to environmental stability and equates his findings to deep sea situations. If this is true, our subaerial deep sponges are living in conditions closer to reef top species than those of abyssal depths, though sponges clearly dominate the biomass of subaerial trawlings, in agreement with Dayton's work on Antarctic sponges.

CONCLUSIONS

From this preliminary study, the following conclusions can be drawn:

1. Subaerial zones shelter a wide range of species and genera of sponges that are quite distinct to those found on reef tops.

2. Deep sponges are generally as toxic as reef species. In such environments, predation and competitive pressures are poorly known but different to those prevailing in coral reefs where photosynthesis plays a determining role.

3. Structural defenses are widespread in sponges from both biota, but hard shells and needle-like sclerites are favored by deep species, whereas shallow sponges often are tough and fibrous as well as malodorous.

ACKNOWLEDGEMENTS

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