

COMPARATIVE ICHTHYOTOXICITY OF SHALLOW AND DEEP WATER SPONGES OF NEW CALEDONIA

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ABSTRACT

A survey of comparative ichthyotoxicity in marine sponges from shallow and deep water in the southern province of New Caledonia was conducted. Test fish *Gambusia affinis* (Vertebrata. Pisces) were exposed to aqueous macerates of 30 shallow water and 30 deep-water sponges. Mortality counts and behavioural changes were recorded over twelve hours, readings taken on a geometric time scale. The responses exhibited by the fish ranged from rapid mortality and varying level of distress and narcotization, to effects indistinguishable from controls. Sponges were classified into three major toxicity groups: 100% lethal, toxic (<100% lethal, with deleterious effects) and non-toxic (indistinguishable from controls). There was little or no overlap in species composition between the shallow and deep-water sponges. Observed toxicity levels varied widely both in the deep and shallow species. The frequency of toxicity was generally equivalent between shallow and deep-water species, although deeper species tended to exhibit a higher degree (intensity) of toxicity. Approximately 80% of species from both depths were found to be toxic. This implies that the two habitats sampled have probably experienced similar degrees of environmental stability through evolutionary time.

INTRODUCTION

As in terrestrial plants, marine invertebrates are known to contain a wide range of secondary metabolites (Feeny 1975, Selover & Crews 1980, Littler *et al.* 1986), particularly in the tropics. Coral reefs have received a great deal of attention from both ecologists and organic chemists because of the high diversity of species and the high incidences of secondary metabolites which occur there (Brown *et al.* 1970, Kittredge *et al.* 1974).

Sponges are among the most common and best chemically studied group of marine animals (see Faulkner, 1984, 1986, 1987). Some ecologists have studied the toxicity of reef sponges in relation to fish predation (Randall & Hartman 1968, Bakus & Green 1974, Bakus & Thun 1979, Green 1977, Bakus 1981). Bakus *et al.* (1986) have reviewed the various roles which secondary metabolites can play in competition for space, anti-fouling, and reproduction. Structural adaptations also play a non-chemically mediated role in feeding deterrence.

Comparative toxicity studies between different geographical areas have also been performed (Bakus 1974, Green 1977, McClintock 1987). These studies included sponge species from tropical, temperate

and antarctic waters, but no bathymetric correlations were recorded. This paper represents the first study of New Caledonian sponges which compares ichthyotoxicity levels of sponges from the shallow lagoon environment with those of the lesser-known deep subreefal slopes.

MATERIALS AND METHODS

Collection of specimens

Thirty shallow water sponges were collected from various reefal zones around Noumea, New Caledonia (22°S, 166°E) between January 1986 and June 1987, at depths ranging from 10 cm to 35 m. Thirty deepwater sponges were dredged from 200-670 m depth on the deep subreefal slopes in the southern provinces of New Caledonia during the same time period. Specimens utilized in toxicity testings were placed in labelled plastic bags and deep frozen. Corresponding specimens were preserved in 70% ethanol and utilized as reference samples for taxonomic determinations.

Toxicity tests

Ichthyotoxicity tests were adapted from techniques developed in studies of soft coral toxicity in the central region of Great Barrier Reef, Australia (Coll *et al.* 1982, Coll & Sammarco 1983, La Barre *et al.* 1986, Sammarco *et al.* 1987; see also Yamanouchi 1955, Bakus & Thun 1979). Aqueous extracts of sponges were prepared by blending 50 grams of frozen tissue with 100 ml of fresh water and centrifuging the macerate at 2,000 rpm for 30 min. The resulting supernatant afforded two portions (50 ml) for the replicate ichthyotoxicity bioassay utilizing *Gambusia affinis* (Baird 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 1 litre of fresh water. Divisions between the replicate compartments were translucent to visually isolate the fish from each other.

Five adult or subadult fish (100 to 300 mg in weight and 19-30 mm in length) were placed in each compartment. Different sized fish were distributed uniformly among test containers. It was assumed that, in general, sexes among the 1200 fish were randomly distributed among test aquaria.

Observations of the status and behaviour of fish were made as follows, in a manner similar to that described by Coll *et al.* (1982):

- Mortality (alive or dead);
- Location (surface, mid-water or bottom);
- Orientation (normal, lateral roll, vertical roll, or both lateral and vertical roll);

- Movement (none, hypoactive, normal, hyperactive);
- Fin activity (none, hypoactive, normal, hyperactive);
- Response to visual stimulus: a sudden shading was caused by blocking the clear faces of the compartments by a black object.

Behavioural patterns were recorded for each fish prior to the addition of extracts and after addition on a geometric time-scale: 22 min, 45 min, 1.5 h, 3 h, 6 h, and 12 h.

Numerical methods

The behavioural data derived from fish subjected to sponge extracts were submitted to computerized pattern-seeking analyses. 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 & 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 summarizing a series of responses, with state 1-3 being relatively normal, and states 6-8 being abnormal.

In the preliminary analysis presented here, a toxicity ranking was established primarily on the basis of mortality count through time. The behavioural data were used to differentiate between sponge species, the extracts of which produced similar mortality counts. Average behavioural state (B) was used in these cases. As no control fish exhibited a mean behavioural state higher than 3.3, 3.5 was used as a conservative cut-off for treatments indistinguishable from controls.

RESULTS

There was no overlap in species composition of sponges between shallow and deep-water sponges. In fact there was no generic overlap between these two depths.

The sponges tested exhibited a wide range of toxicity (table 1.) The sponge species fell into three major ichthyotoxicity groups:

- 1) 100% mortality of test fish within 720 mins; considered to be lethal;
- 2) <100% mortality of tests fish, with test fish exhibiting abnormal behaviour; considered to be toxic, and
- 3) no mortality and behavioural attributes indistinguishable from controls, considered to be non-toxic.

With respect to the first group (table 1), 6 of the 30 shallow species were found to be lethal while 12 of the deeper-water specimens exhibited the same level of toxicity (figure 1). In shallow-water, *Haliclona* sp. 93 and *Ircinia* sp. 59 were found to be extremely toxic, killing all fish in 22 minutes. Species 31 from the deep-water collection yielded a similar response. On the other hand, some sponges such as *Axinyssa* sp. 85

from shallow-water and *Tethya* sp. 27 from deep-water showed a much more delayed response to produce the total mortality in test fish. With respect to the "toxic" group (19 shallow species, 12 deep species) the number of species causing some mortality (<100%) in test fish was the same in shallow (9) and deep-water (9). On the other hand, the number of species causing sublethal but deleterious effects (abnormal behaviour but no mortality) was much higher in the shallow-water group (10) than in the deep-water group (3), in opposition to the trend noted in the group deemed "lethal". The effects observed in this group ranged from 90% mortality after 720 mins in the shallow-water sponge *Callyspongia* sp. 65, and the deep-water sponge *Petrosia* sp. 9, to no mortality and moderately deleterious effects in *Clathria* sp. 87 (shallow) and *Jaspis* sp. 39 (deep-water).

Approximately equal numbers of shallow and deep-water sponge species (5-6) produced test results indistinguishable from controls.

We were unable to demonstrate any significant difference between the frequency of occurrence of toxicity in shallow vs. deep-water sponges, whether considered as 3 groups (as above) or 2 (non-toxic vs. any deleterious effects) ($p > 0.05$, G-test of independence). It is important to note however, that twice as many "lethal" species were found in the deep as opposed to the shallow habitat (figure 1).

Altogether, 80% of the samples caused abnormal behaviour in *Gambusia 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 in all fish and 25% of the sponges killed all fish with some delay.

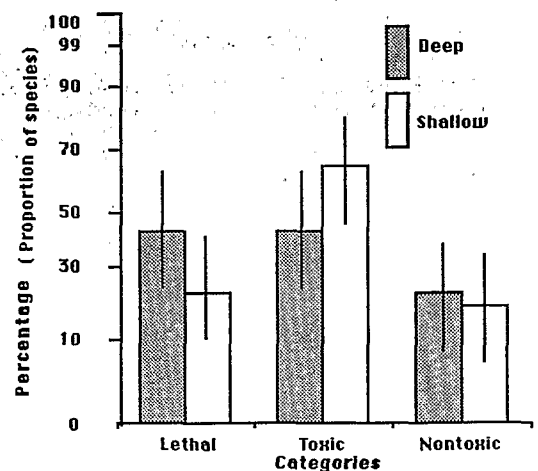


Figure 1. Distribution of sponge species within toxicity groups.

DISCUSSION

We have determined the relative toxicities of 60 species of common sponges (30 shallow-dwelling and 30 deep sub-reefal) representing 42 genera. A wide range of responses was observed in test

TABLE 1. Ichthyotoxicity Results

SHALLOW-WATER SPONGES								DEEP-WATER SPONGES									
Species	Specimen	Mortality Count						B	Species	Specimen	Mortality Count						B
	No.	22	45	90	180	360	720		No.	22	45	90	180	360	720		
Lethal group (100% mortality after 720 mins)								Lethal group (100% mortality after 720 mins)									
<i>Haliclona</i> sp.	93	10	10	10	10	10	10	<i>Undetermined</i> sp.	31	10	10	10	10	10	10		
<i>Ircinia</i> sp.	59	10	10	10	10	10	10	<i>Phloeodictyon</i> sp.	55	4	10	10	10	10	10		
<i>Undetermined</i> sp.	113	4	10	10	10	10	10	<i>Podospongia</i> sp.	3	0	10	10	10	10	10		
<i>Toxochalina</i> sp.	111	0	0	7	10	10	10	<i>Reidia</i> sp.	47	0	10	10	10	10	10		
<i>Damiriana</i> sp.	63	0	0	0	10	10	10	<i>Reidia</i> sp.	33	0	6	10	10	10	10		
<i>Axinyssa</i> sp.	85	0	0	0	1	8	10	<i>Phloeodictyon</i> sp.	25	0	4	10	10	10	10		
Toxic group (<100% mortality: abnormal behaviour)								Toxic group (<100% mortality: abnormal behaviour)									
<i>Callyspongia</i> sp.	65	0	0	0	0	0	9	<i>Xestospongia</i> sp.	7	0	4	10	10	10	10		
<i>Dendrylla</i> sp.	73	0	0	0	0	0	9	<i>Neosiphonia</i> sp.	49	0	0	0	10	10	10		
<i>Undetermined</i> sp.	83	0	0	0	3	5	8	<i>Undescribed</i>	1	0	0	0	0	10	10		
<i>Dendrylla</i> sp.	97	0	0	0	0	0	6	<i>Myxillid</i> sp.									
<i>Mycale</i> sp.	103	0	0	0	4	5	5	<i>Pheronema</i> sp.	15	0	0	0	2	5	10		
<i>Haliclona</i> sp.	109	0	0	0	0	0	4	<i>Tethya</i> sp.	27	0	0	0	0	0	10		
<i>Heteronema</i> sp.	71	0	0	0	0	0	4	<i>Undetermined</i> sp.	11	0	0	0	0	0	10		
<i>Cliona</i> sp.	61	0	0	0	0	2	3	Toxic group (<100% mortality: abnormal behaviour)									
<i>Cliona</i> sp.	91	0	0	0	0	0	0	<i>Petrosia</i> sp.	9	0	0	0	0	0	9		
<i>Cliona</i> sp.	119	0	0	0	0	0	6.71	<i>Cladocroce</i> sp.	13	0	0	0	1	4	8		
<i>Spirastrella</i> sp.	75	0	0	0	0	0	5.00	<i>Corallistes</i> sp.	53	0	0	0	1	2	8		
<i>Undetermined</i> sp.	115	0	0	0	0	0	4.42	<i>Geodia</i> sp.	43	0	0	0	0	0	3		
<i>Echinochalina</i> sp.	101	0	0	0	0	0	4.43	<i>Stelletta</i> sp.	41	0	0	0	0	0	3		
<i>Siphonochalina</i> sp.	89	0	0	0	0	0	4.28	<i>Geodia</i> sp.	45	0	0	0	1	1	1		
<i>Haliclona</i> sp.	67	0	0	0	0	0	4.00	<i>Corallistes</i> sp.	19	0	0	0	0	0	1		
<i>Ircinia</i> sp.	69	0	0	0	0	0	4.00	<i>Stelletta</i> sp.	37	0	0	0	0	0	1		
<i>Unidentified</i> sp.	117	0	0	0	0	0	3.71	<i>Stylotella</i> sp.	105	0	0	0	0	0	1		
<i>Unidentified</i> sp.	77	0	0	0	0	0	3.71	<i>Stylotella</i> sp.	35	0	0	0	0	0	5.00		
<i>Clathria</i> sp.	87	0	0	0	0	0	3.57	<i>Undetermined</i> sp.	57	0	0	0	0	0	4.14		
Non-Toxic group (indistinguishable from controls)								Non-Toxic group (indistinguishable from controls)									
<i>Cliona</i> sp.	79	0	0	0	0	0	3.43	<i>Undetermined</i> sp.	29	0	0	0	0	0	3.14		
<i>Clathria</i> sp.	81	0	0	0	0	0	2.86	<i>Geodia</i> sp.	51	0	0	0	0	0	2.71		
<i>Undescribed</i> <i>Axinellid</i>	95	0	0	0	0	0	2.86	<i>Geodia</i> sp.	23	0	0	0	0	0	2.28		
<i>Chondropsis</i> sp.	107	0	0	0	0	0	2.28	<i>Pheronema</i> sp.	17	0	0	0	0	0	2.14		
<i>Spinoseella</i> sp.	99	0	0	0	0	0	2.28	<i>Corallistes</i> sp.	21	0	0	0	0	0	1.86		
Controls (30x)		0	0	0	0	0	<3.50	<i>Ragadrella</i> sp.	5	0	0	0	0	0	1.71		
								Controls (30x)		0	0	0	0	0	<3.50		

organisms, in accordance with similar studies on sponge toxicities (Bakus & Thun 1979, Bakus 1986). Effects were observed ranging from almost immediate death to mildly abnormal behaviour. Some examples showed more or less intense narcotization, where alertness and orientation were affected. Other examples showed alternations between hyperactive and sedate phases, with numerous individual variations (e.g. defecation, body contractions, strong hyperactive response to sudden light changes, spawning by gravid females, escape response, etc., all indicative of stress. With respect to these lethal and sublethal effects, pharmacological tests currently underway in our laboratories correlate well with the behavioural responses observed in this study (S.C.L.B. & D.L.).

Sponges provide the greatest diversity of secondary metabolites of any single phylum examined to date (Faulkner 1984, 1986, 1987). The occurrence and distribution of these compounds has been used successfully in the delineation of certain taxa within the Porifera (Bergquist & Wells 1980). Such techniques have also been used for other marine invertebrates (e.g. Gerhart 1983). Some of the observed variance between species may be due to several factors. The concentration of a single toxic metabolite may vary by several orders of magnitude, between closely related congeneric species, or fluctuate seasonally in a given intraspecific population (A. Ahond, pers. comm.), but the same metabolite is rarely found across unrelated genera.

Although no quantitative data were collected on morphology, highly toxic individuals tended to lack effective structural armament, whereas mildly or non-toxic species tended to be protected by calcareous shells, needle-sharp spicules (in deep-water species) or tough, leathery tissue (in shallow-water species). Little is known, however, of predation and other selection pressures at such depths. Nor has the correlation between the occurrence of one type of defense and the absence of another been established for this group (but see Sammarco et al., 1987).

The fact that there was no overlap whatsoever in either genera or species between the two depths sampled implies that the study areas indeed represented two totally different communities from very different physical regimes. Therefore, any similarity in toxicity levels would have important evolutionary implications concerning the general stability of the two communities.

Analyses of the distribution of ichthyotoxicity were unable to demonstrate any significant difference between shallow and deep-water species of sponges. Twice as many "lethal" sponges were found in deep-water as opposed to shallow-water, but this imbalance was insufficient to cause a significant difference in overall toxicity between the two states.

The implications of this finding are important. Deeper oceanic waters have generally been considered to represent a stable environment through evolutionary time (Sanders 1968). They have certainly been more stable than shallower environments, which have experienced numerous sea level changes and temperature fluctuations causing mass extinctions (Stanley 1984). In addition, the Western Pacific appears to have been more stable

than other geographic regions, as evidenced by higher species richness, and more intense biological interactions with respect to predation and competition (Vermeij 1978). Thus, the lack of difference in general frequency of toxicity between deep and shallow-water sponges here implies that these two environments have most likely been approximately equally stable through evolutionary time. We would predict that this would not be the case for the tropical Western Atlantic (Stanley 1984, Sammarco 1985, 1987), tropical Eastern Pacific (Risk et al. in press) or the temperate regions; i.e. we would expect to find differences in toxicity between sponges from deep and shallow water in these environments. McClintock (1987) has found high (56%) toxicity levels in Antarctic sponges, an area considered to be highly stable (Dayton 1974).

The overall frequency of toxicity observed in these New Caledonian sponges (approx. 80%) is comparable to those determined on the Great Barrier Reef for sponges (approx. 60%) by Bakus (1981) who sampled generally from shallow waters. It is significantly higher, however, than the levels determined for alcyonaceans (approx. 50%) also from the Great Barrier Reef (Coll et al. 1982, Coll & Sammarco 1983).

CONCLUSIONS

The major findings of this study are as follows:

1. There was little or no overlap in species composition between shallow and deep-water sponges of New Caledonia.
2. Toxicity varied widely between sponge species found both in shallow and deep water.
3. Toxic deep-water sponges occurred generally as frequently as toxic shallow reef species, although the degree of toxicity ("lethality") was more commonly observed in deep-water species.
4. Approximately 80% of both shallow and deep-water species were found to be toxic.
5. Similarity in toxicity levels between shallow and deep-water sponges in this study implies that the two habitats from which the sponges were drawn have probably experienced similar degrees of environmental stability through evolutionary time.

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