Proceedings (Poster) VIth International Coral Reef Symposium Townsville, Austr. 8-12 VIII 1988

COMPARATIVE ICHTHYOTOXICITY OF SHALLOW AND DEEP WATER SPONGES OF NEW CALEDONIA

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ABSTRACT

We have conducted a survey of toxicity distribution with depth of common marine sponges of the Southern province of New Caledonia.
Test fish Cambusia 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 behavioural 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 behavioural patterns (from 8 computer-defined patterns) were classified into 6 toxicity groups, falling into 3 broad categories : 100% lethal, harmful to toxic, and non-toxic.
Eighty percent 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 (Feeny, 1975 ; Selover and Crews, 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 affords a surprisingly large number of organic molecules of the same types as encountered on land (Brown et al., 1970 ; Kittredge et al., 1974).
Sponges are among the most common and the best studied group by chemists (Faulkner, 1987). Often independantly, 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).
Feeding 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 ; Bakus, 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 subreefal slopes. Although we have extensively relied on techniques devised by us on studies of Great Barrier Reef alcyonarians, 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 reefal zones around Noumea, New Caledonia (166°S 22°E) between January 1986 and June 1987, at depths ranging from 10 cm to 35 meters of depth. 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. Identifications were made by Prof. Claude Levi of the Museum d'Histoire Naturelle de Paris[1], and illustrated references compiled by Pierre Laboute of ORSTOM at Noumea[2].

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 developped 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 mls of ffresh water and centrifuging the macerate at 2,000 rmp for 30 mins. The resulting supernatant afforded two portions (50 mls) for the replicate ichthyotoxicity bioassay utilizing Cambusia 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 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 mls 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 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 at the same time by black surfaces.

[1,2] q.v. Aknowledgements.

- Mortality (alive or dead).

Behavioural patterns were recorded for each fish at t_0 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 behavioural data derived from fish subjected to sponge extracts were submitted 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 summariling a serie of responses .

The original data where then analysed 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.

Response code	Location	Orientation	Movement	Fin activity	Response to visual stimulation
	Key to response code :				
A	surface	normal	none	none	none
B	mid	lateral roll	hypoactive	hypoactive	hypoactive
C	bottom	vertical roll	normal	normal	normal
D		both	hyperactive	hyperactive	hyperactive
	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	5.698
B	1.116	1.279	3.884	4.047	3.581
C	6.535	0.070	0.395	0.674	0.605
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	7.551
D	0.000	0.000	0.184	0.265	0.939
	State 4				
A	8.826	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	3.456	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	9.992	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	9.951	0.343	0.255	0.088
B	2.873	0.039	0.461	0.216	0.225
C	3.833	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).

Genus	Specimen number	Behavioural state time (min) 0	22	45	90	180	360	720
Group I : 3 sponges, all fish dead at 22 min								
Haliclona	93	1	8	8	8	8	8	8
undetermined	31	1	8	8	8	8	8	8
Ircinia	59	1	8	8	8	8	8	8
Group II : 15 sponges, all fish dead at 720 min								
Calcarea	113	1	8	8	8	8	8	8
Phloeodictyon	55	1	8	8	8	8	8	8
Podospongia	3	3	8	8	8	8	8	8
Xetospongia	7	1	5	8	8	8	8	8
Phloeodictyon	25	1	4	8	8	8	8	8
Reidia	47	1	3	8	8	8	8	8
Reidia	33	1	4	4	8	8	8	8
Toxochalina	111	2	4	4	8	8	8	8
Damiriana	63	2	6	4	7	8	8	8
Neosiphonia	49	3	3	3	2	8	8	8
Myxillidae	1	1	4	4	7	6	8	8
Axynissa	85	2	4	5	5	4	8	8
Pheronema	15	1	6	7	7	7	7	8
undetermined	11	2	2	4	5	4	7	8
Tethya	27	2	2	1	4	4	4	8
Group III : 17 sponges, survivors very ill								
Callyspongia	65	1	6	4	8	8	7	8
Dendrylla	73	1	4	7	7	7	7	8
Petrosia	9	3	6	4	7	7	5	8
Corallistes	53	3	4	4	4	7	7	8
undetermined	83	3	1	3	4	7	7	8
Cladocroce	13	3	3	1	1	4	7	8
Mycale	103	3	6	5	6	7	7	7
Dendrylla	97	3	6	4	5	5	4	7
Haliclona	109	2	7	4	4	7	7	4
Cliona	61	3	7	7	7	7	8	7
Heteronema	71	1	6	4	4	6	4	7
Stelleta	41	3	3	4	6	6	5	7
Geodia	43	3	1	3	3	3	2	5
Cliona	91	3	2	6	6	6	6	2
Stelleta	37	1	4	5	5	5	5	5
Geodia	45	1	4	4	2	3	2	2
Corallistes	19	3	1	3	2	2	2	2
Group IV : 10 sponges, all fish ill								
Cliona	119	3	7	8	8	7	7	7
Spirastrella	75	3	4	4	6	6	6	6
undetermined	115	1	3	3	6	6	6	6
Haliclona	67	3	1	4	4	4	6	6
Stylotella	35	2	7	5	5	6	5	5
Ircinia	69	3	2	6	4	5	4	4
Liesina	117	3	1	3	2	5	7	5
Echinochalina	101	3	2	6	6	6	6	2
Stylotella	105	3	4	4	3	1	3	2
Geodia	51	1	1	4	2	3	2	6
Group V : 11 sponges, 1 multiple control, some fish ill								
Cliona	79	1	4	4	7	6	4	4
Jaspis	39	2	4	4	4	4	5	5
Siphonochalina	89	4	4	6	6	4	4	2
Clathria	87	1	4	4	4	4	4	4
undetermined	77	3	1	4	6	4	6	2
undetermined	57	3	6	6	4	3	4	3
Axinellidae	95	1	6	3	1	6	2	1
undetermined	29	3	4	4	4	1	2	4
Clathria	81	1	3	6	3	3	3	1
control	42,92,98, 104,118	3	3	3	2	4	2	6
Corallistes	21	1	2	2	1	2	1	4
Spinosella	99	3	1	1	4	1	4	2
Group VI : 4 sponges, 22 multiple controls, no lasting effect if any								
control	2,114	1	3	3	3	3	3	7
control	62,120	1	2	3	3	1	2	6
control	8,22	1	1	1	1	1	1	4
control	66,94,110	3	3	2	2	1	1	4
control	30,32	3	2	2	1	3	3	4
control	34,36,38,40	2	1	1	2	2	2	4
control	74,82,96	3	2	1	3	1	1	3
control	50,52,56,58	1	3	3	1	3	1	3
control	84,86	3	3	1	2	3	2	1
Pheronema	17	3	3	3	2	1	1	2
control	4,14	1	1	1	1	1	1	2
Regadrella	5	3	1	3	1	2	1	1
control	12,28	2	3	1	3	3	3	1
control	44,48,54,60	1	3	3	3	3	3	1
control	16,26	1	3	2	3	3	3	3
control	102,116	1	1	2	3	3	3	3
control	78,88,90,100	1	1	3	3	3	3	3
control	64,68,70,76,80	1	3	3	3	3	3	3
control	46	1	3	3	1	3	3	3
control	6	1	1	3	3	1	3	3
control	72	3	1	1	3	1	1	3
control	18,20	1	1	2	1	1	3	2
Chondropsis	107	3	1	1	3	3	3	2
Geodia	23	3	1	1	3	3	3	2
control	112	2	1	1	2	1	2	2
control	10,24,106,108	3	3	3	3	3	3	3

Table 2. *Gambusia affinis*. Behavioural responses through time during exposure to crude extracts of each of 60 sponges. Each entry represents the responses of 10 fish as summarized by the behavioural states (1-8) defined in table 1. Groups I - VI are determined arbitrarily, using mortality, severity of toxicity and changes through time as ranking criteria. Specimens with odd numbers are controls, specimens with even numbers 2 to 60 are shallow water sponges and 62 to 120 are deep water sponges.

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).

State 1 characterizes dead or dying fish, mostly at the bottom, lying on their sides, motionless and totally irresponsive to external light changes.

States 2 and 3 described sublethal to lethal conditions, with alternation of deep aloofness 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, nose-dive or 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 hypoactive 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 behaviour without loss of orientation during swimming, ie. 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 hypersensivity to sudden light changes. Defecation often immortant 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 behaviour : in state 8 fish are not mobile but their orienta-

TOXICITY CATEGORIES AND GROUPS

	100 % lethal		harmful to toxic		non toxic	
GENERA REPRESENTED	I	II	III	IV	V	VI
Haliclona	1		1	1		
* undetermined 31	1					
Ircinia	1			1		
Calcarea		1				
* Phloeodictyon		2				
* Podospongia		1				
* Xestospongia		1				
* Reidia		2				
Toxochalina		1				
Damiriana		1				
* Neosiphonia		1				
* Myxillidae		1				
Axynissa		1				
* Pheronema		1				1
* undetermined 11		1				
* Tethya		1				
Callyspongia			1			
Dendrylla			2			
* Petrosia			1			
* Corallistes			2		1	
undetermined 83			1			
* Cladocroce			1			
Mycale			1			
Cliona			2	1	1	
Heteronema			1			
* Stelleta			2			
* Geodia			2	1		1
Spirastrella				1		
undetermined 115				1		
* Stylotella				2		
Liesina				1		
Echinochalina				1		
* Jaspis					1	
Siphonochalina					1	
Chlathria					2	
undetermined 77					1	
* undetermined 57					1	
Axinellidae					1	
* undetermined 29					1	
Spinosella					1	
* Regadrella						1
Chondropsis						1

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_0 before addition of the extracts into the tanks, and can be regarded as the results of normal interactions between fish and 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 h). A wide range of genera (Table 3) were represented in the 100% lethal groups, two thirds of which were from deep bottom trawlings : Ircinia, Phloeodictyon (2 species), Podospongia, Xestospongia Reidia (2 species), Neosiphonia, Pheronema, Thetya, 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 toxicites (in progress). Except for Pheronema, these genera were confined to groups I and II. Shallow genera included Haliclona, an unknown Calcarea, Toxochalina, Damiriana, Axynissa and a member of the Myxillidae family.
Groups III and IV clearly induced severely abnormal behaviour in the test organism, 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 Petrosia, Corallistes (2 species), Cladocroce, Stelleta (2 species) and Geodia (3 species). Shallow specimens included Haliclona (2 species), Callyspongia, Dendrylla (2 species), Mycale, Cliona (3 species), Heteronema, Spirastrella, Liesina, Echinochalina and two undetermined entities.

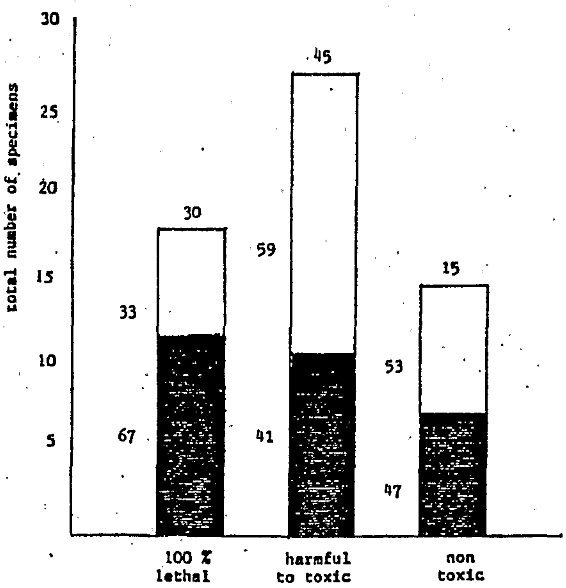

Fig. 1 - Entries showing percentages per category of toxicity (top) and relative percentages within each category between deep (dark) and shallow (light) boxes.

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 : Jaspis, Regadrella and two undetermined genera. Non toxic shallow genera included Siphonochalina, Clathria, Spinosella, Chondropsis and axinellid and one undetermined sponge, plus Pheronema 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) snowed 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 commun 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 Thun, 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 stabilized symptoms, others producing gradual or fluctuating effects. This variability was reflected qualitatively. Some examples showed more or less intense narcotization 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 (eg. strong defecations, body contacts, strong hyper-reactivity to sudden light changes, bithgiving 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, independantly of subtle variations in "mild" behavioural states. Synergetic 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 activites exhibited by the sponges in pharmacological tests currently underway in our laboratories matches 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 congeneric species, or fluctuate seasonally in a given intraspecific population (Alain Ahond, pers. comm.) but the same metabolite is rarely found across unrelated genera. We indeed found that 37 of the genera tested (88%) 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 (*Phloeodictyon* 25 and 55 ; *Reidia* 47 and 33 ; *Geodia* 43 and 45). In general, 100% lethal sponges were found in deep trawlings (63% of the species, ie. 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 subreefal bottoms.

As for shallow sponges, highly toxic individuals tended to lack effective structural armament, 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 Bakus (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 sponge 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 antartic sponge populations are attributable to environmental stability and equates his findings to deep sea situations. If this is true, our subreefal deep sponges are living in conditions closer to reef top species than those of abyssal depths, though sponges clearly dominate the biomass of subreefal trawlings, in agreement with Dayton's work on Antartic sponges.

CONCLUSIONS

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

1. Subreefal 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 favoured by deep species, whereas shallow sponges often are tough and fibrous as well as malodorant.

ACKNOLEDGEMENTS

We wish to thank Mrs Bargibant, Menou and Tirart of ORSTOM for extensive SCUBA collection and dredgings, M. Laboute of ORSTOM for photographic records, and above all, Professor Claude Levi of Museum d'Histoire Naturelle de Paris, 32 Rue Buffon, Paris 75006, for taxonomic identifications and Ecole Pratique des Hautes Etudes at the Museum for proposing a travel grant to S. La Barre.

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