MARINE RESEARCII, 1995, 4(1): 33-46

BIOACTIVITY OF GORGONIANS (SUB-CLASS OCTOCORALLIA) IN SINGAPORE: PRELIMINARY STUDIES

Nigel K.C. Goh[1], T.S. Sim[2] and L.M. Chou[1]
[1]Dept. of Zoology, National University of Singapore
Kent Ridge, Singapore 119260 and
[2]Dept. of Microbiology, National University of Singapore
Kent Ridge, Singapore 119260

ABSTRACT

Extracts of six species (from four families) of gorgonians collected from Singapore coral reefs were tested for toxicity against representative microorganisms (gram positive and gram negative bacteria, a yeast, and a mould), the brine shrimp (*Artemia salina*) and the goldfish (*Carassius auratus*). All extracts tested showed some antimicrobial activity at concentrations equivalent to 0.5g of natural gorgonian tissue (based on sample wet weights), while five were found to be toxic to *A. salina* (from as low as 1000 ppm) and six (lowest toxic concentration, 2500 ppm) to *C. auratus*. Behaviour modifications were observed in *C. auratus* on addition of extracts, even at non-lethal concentrations. This is the first report of bioactivity in gorgonian corals from Singapore.

INTRODUCTION

The knowledge that extracts of certain plants and animals possess biologically important properties has been known since antiquity. For the simple reason of accessibility, the vast majority of useful extracts were initially of terrestrial origin (Scheuer, 1988). For example, the natives of the Amazon Basin used the toxic exudate from the "poison arrow frogs" to tip their darts; penicillin was derived from the mould *Penicillium*, and an antimalarial extract was obtained from the bark of the *Cinchona*.

Bioactivity in at least some species of gorgonians has also been known for a long time. The ancient Chinese recognized and used the medicinal properties of the gorgonian *Melitodes (=Melithaea) squamata* (South China Sea Institute of Oceanology, 1978; in Scheuer, 1988). Red coral (probably the gorgonian *Corallium rubrum* or a closely related species) was used as a major ingredient in numerous potions and decoctions by the ancient Greeks and passed on to the early European cultures (Hickson, 1924). Burkholder & Burkholder (1958) reported on the antimicrobial activity of gorgonians from the Caribbean. They were probably the first to publish scientifically on the bioactivity of gorgonian corals. Kim (1994) recently found antibacterial activity in extracts of eight gorgonians from Panama. Out of 901 compounds derived from marine flora and fauna (Kennish, 1989), 58 were extracted from gorgonians. Some notable examples include the first discovery of a pros-

taglandin from a non-mammalian source, and in commercially harvestable amounts (Weinheimer & Spraggins, 1969). Crassin acetate, first extracted from *Pseudoplexaura* spp., was found to inhibit P388 leukemia in vivo (Weinheimer & Matson, 1975) while lophotoxin, isolated from gorgonians of the genus *Lophogorgia*, causes ataxia, paralysis, severe respiratory depression and death (Fenical *et al.*, 1981). Anti-inflammatory activity has been reported for many novel terpenoid compounds isolated from gorgonians (Look *et al.*, 1985, 1986; Fenical, 1987; Look & Fenical, 1987; Groweiss *et al.*, 1988; Shin *et al.*, 1989; Kobayashi *et al.*, 1991; Pordesimo *et al.*, 1991), with activity in several of these compounds exceeding that of indomethacin, a clinical drug used against inflammation.

The evolutionary success of Indo-Pacific octocorals, including gorgonians, has been attributed in part to the occurrence of toxic secondary metabolites which minimise predation, increase competitiveness and aid in reproduction (Sammarco & Coll, 1988). In particular, these metabolites deter feeding in invertebrates (Fenical & Pawlik, 1991; Gerhart, 1986; Harvell & Fenical, 1989; Lasker & Coffroth, 1988; Lasker *et al.*, 1988; Van Alstyne & Paul, 1992; Vreeland & Lasker, 1989) and fish (Fenical & Pawlik, 1991; Harvell *et al.*, 1988; Harvell & Fenical, 1989; Lasker, 1985; Pawlik *et al.*, 1987; Sammarco *et al.*, 1987).

The gorgonian fauna in Singapore is diverse and relatively abundant (Goh, unpublished data), with more than thirty species recognised. Compared with the 150 or more species of scleractinians (hard corals) found on Singapore reefs, the screening of gorgonians for bioactivity appears to be a poor choice. However, little or no predation by coral-livorous fish has been observed on them in the field, pointing to the possibility of toxic metabolites within the tissue of these invertebrates. This is despite the fact that octocorals, in comparison with scleractinians, are a potentially rich source of protein, fat, and carbohydrates (Coll, 1981). A review by Tursch *et al.* (1978) also did not reveal significant levels of secondary compounds in the Scleractinia. Most gorgonian species are easily accessible by SCUBA in Singapore, in contrast to other reefs in the region (Goh & Chou, 1994). This facilitates their collection and hence the study of their bioactivities. They also have relatively high rates of growth and regeneration (Goh, unpublished data), allowing the possibility of sustainable harvesting of these organisms if useful metabolites are found.

To date, none of the gorgonians in Singapore has been tested for any form of bioactivity, nor have their chemical structures been elucidated. The present study is of a preliminary nature, with the objective of mapping the occurrence of bioactivity in crude extracts of gorgonians in Singapore using simple bioassays. This will enable future workers to narrow down the search for novel bioactive compounds to species that have shown some activities in these assays. However, it must be emphasized that the bioassays carried out are by no means exclusive. It is likely that useful species may not be detected if screening is dependent on such limited bioassays.

MATERIAL AND METHODS

Collection

Six commonly found species were tested for bioactivity. These were: from the Subergorgiidae, *Subergorgia suberosa* and *S. mollis,* from the Melithaeidae, *A. robusta* from the Ellisellidae, *J. (Dichotella) gemmacea* and *Ctenocella* (Verrucella) sp. A; and from the Plexauridae, *Echinogorgia* sp. E. Voucher specimens of all species tested are lodged in the Zoological Reference Collection of the National University of Singapore. Colonies of the gorgonians tested were hand-collected from reefs south of the main island of Singapore using SCUBA. Each species was collected from only one reef site to prevent inter-locality differences to complicate results. Any debris attached to the colonies was removed immediately at collection. When brought to the surface, metabolic activity was arrested by pouring liquid nitrogen over the collections of each species (hereinafter known as samples). These samples were then stored in ice until transferred to a laboratory freezer at-30°C.

Extract Preparation

Samples were ground using a food processor and the weight of the ground sample noted. Samples were completely immersed in acetone, and left overnight. The mixture of sample and solvent (acetone) was then filtered under vacuum. The residue was re-extracted in fresh acetone overnight while the filtrate was stored. This process was repeated so that a total of three extractions were performed. The three resulting filtrates were combined and the acetone removed by evaporation under vacuum using a rotary evaporator, giving the crude extracts.

Anti-microbial Activity

The six extracts were tested for toxicity against representative Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli)* bacteria, a yeast (*Saccharomyces cerevisiae*) and a mould (*Aspergillus* sp.) using the disc assay method. An aliquot (equivalent to 0.5g wet weight of tissue) of each test extract was spotted onto a filter paper disc (Difco concentration discs, 1/4", 1599-33) and allowed to air-dry. Three replicate discs were used for each test extract. Discs were placed on a lawn of the growing microbe and positive assays were measured in terms of the diameter of a clear zone of inhibition of microbial growth around the disc after overnight incubation at the appropriate temperature (bacteria: 37°C; yeast, mould: 28°C). Controls using seawater and acetone were also included.

Brine Shrimp Toxicity

Artemia salina cysts (Argentemia, Argent Chemical Laboratories, Redmond, WA, USA) were hydrated in a petri dish with artificial seawater and placed under a lamp. Hatching occurred in about 12 hours. Sets of ten freshly hatched nauplii were collected using a Pasteur pipette and placed in separate wells of a multi-welled container. Test solutions were made up to 2ml and added to each of three wells giving a sample size of 30 brine shrimp for each test solution. Both positive (various concentrations of potassium dichromate solution) and negative (artificial sea-

water) controls were also set up.

Mortality was scored in each well after 6h (acute exposure) and 24h (chronic exposure). Nauplii were counted under a microscope. A nauplius was considered dead if it lay immobile at the bottom of the well or if the appendages, e.g., the antennae, antennules and mandibles were inactive. Results were analysed using the Reed-Muench method described in Teng (1993).

Goldfish Toxicity

Carassius auratus (5-6 cm total length), used in this bioassay, were obtained commercially and allowed to acclimatize to laboratory conditions for 2-3 days. Six randomly selected fish were put into each test aquarium containing 3000 ml of dechlorinated water and the test extract. Gentle aeration was maintained throughout the duration of the experiment. Three concentrations (2500, 5000 and 75000 ppm) of each extract were tested. A control of the same volume of dechlorinated water was also set up.

As in the *A. salina* assay, mortality was recorded after acute (6h) and chronic (24h) exposure to the test solutions. Analysis was again based on the Reed-Muench method.

Behaviour Modification in Goldfish

Qualitative observations were made in terms of the behaviour of the fish every 15 minutes for the first hour after addition of the test solutions.

RESULTS

The gorgonian extracts were diverse in terms of the inhibition spectrum against the selected bacteria and eukaryotic test microbes. None of the extracts was toxic to all four classes of microorganisms tested (Table 1). Extracts from the subergorgiids (*S. suberosa* and *S. mollis*), the plexaurid, *Echinogorgia* sp. E, and the ellisellid *J.(D.)* sp. *(D.)* sp. *cf. gemmacea* were found to show broad-spectrum inhibitory effects in that both Gram positive and Gram negative bacteria were inhibited. *A. robusta* and ctenocella (*Verrucella* sp. A were found to be inhibitory to Gram positive *B. subtilis* but not to Gram negative *E. coli*. Among the six gorgonian species examined, only the two ellisellid species showed anti-eukaryotic effects against *S. cerevisiae*. The mould, *Aspergillus* sp. proved resistant to extracts of all six species tested. Under the same test conditions, sea-water and acetone did not inhibit the microorganisms tested.

At lower concentrations (1000, 5000 and 7500 ppm), acute (6h) exposure of *A. salina* caused less than 4% mortality, even in the most potent extract (Fig.1). At 10000 ppm, mortality after 6h ranged from 0% (*J. D.)* sp. *cf. gemmacea* and *Echinogorgia* sp. E) to 19.35% (*S. suberosa*). Chronic (24h) exposure of *A. salina* (Fig. 2) at extract concentrations of 1000 ppm and 5000 ppm did not cause significant mortality (< 8%). At 7500 ppm, maximum mortality due to the extracts was 22.41% (*J. (D.) cf. gemmacea*), while at 10000 ppm, *S. suberosa* was the most toxic, causing 50% mortality of *A. salina*. A negative control consisting only of filtered artificial seawater caused no mortality, while mortality in the positive control (potassium dichromate) is recorded in Table 2.

At the lowest extract concentration (2500 ppm) tested on the goldfish no

(2500 ppm) tested on the goldfish no mortality was recorded after 6h of exposure (Fig. 3). At 5000 ppm, only *S. suberosa* (50%) and *J. (D.)* sp. *cf. gemmacea* (28.57%) extracts caused goldfish mortality. Percentage mortality at 7500 ppm extract concentration ranged from 0% (*A. robusta* sp. A and *Echinogorgia* sp. E) to 100% (*S. suberosa*). The extract of *J. (D.)* sp. *cf. gemmacea* also potent at

Table 1. Antimicrobial activity of crude extracts from six gorgonian species

Species	Inhibition diameter (mm) (mean of 3 replicates each)			
	B. subtilis	*E. coli*	*S. cerevisiae*	*Aspergillus* sp.
Subergorgiidae				
S. suberosa	10.3	11	0	0
S. mollis	12	9.5	0	0
Melithaeidae				
A. robusta	9	0	0	0
Ellisellidae				
J. (Dichotella) sp. *cf. gemmacea*	10.3	9	16.7	0
Ctenocella (Verrucella) sp. A	9.7	0	14.3	0
Plexauridae				
Echinogorgia sp. E	10.3	12	0	0
Controls				
Seawater	0	0	0	0
Acetone	0	0	0	0

Table 2. Percent mortality of freshly hatched brine shrimp larvae to potassium dichromate solutions (positive control)

Conc.(ppm)	Percent Mortality	
	Acute (6h) Exposure	Chronic (24h) Exposure
1000	100	100
800	100	100
600	100	100
400	100	100
80	50.79	100
60	7.87	100
40	0	100
20	0	91.84

this concentration, causing 87.5% mortality, while *Ctenocella (Verrucella)* sp. A killed 33.33% of the goldfish. *S. mollis* was also toxic at this concentration, causing 16.67% mortality. As expected, chronic (24h) exposure of the goldfish to the extracts elicited a generally greater response (Fig. 4). However, as in the acute exposure, no mortality was recorded in *A. robusta* and *Echinogorgia* sp. E. extracts at all concentrations. At 2500 ppm, *S. suberosa* was the only toxic extract, causing 16.67% mortality. *S. suberosa* and *J. (D.) cf. gemmacea* extracts caused 100% and 83.33% mortality, respectively, at 5000 ppm, while *C. (Verrucella)* sp. A killed 33.33% of the goldfish. Maximum mortality (100%) was recorded at 7500 ppm after 24h exposure to extracts of *S. suberosa, J. (D.)* sp. *cf. gemmacea* and *C. (Verrucella)* sp. A. In comparison, prolonged exposure to the extract of *S. mollis* did not cause an increase in mortality with respect to the 6h exposure. No mortality was recorded in the control.

All extracts caused some behaviour modification in goldfish within the first hour of exposure (Table 3). Reduced activity resulted from extracts of *S. sub-*

Table 3. Behaviour of the goldfish during the first hour after exposure to extracts;
- : normal activity, breathing and orientation; RA : reduced activity;
GA : gulping for air at surface; DO : disoriented

Species	Extract Conc.(ppm)	Behaviour throughout first hour			
		15 min	30 min	45 min	60 min
S. suberosa	2500	-	-	RA	RA
	5000	-	RA	RA	RA
	7500	-	RA, DO	DO	DO
S. mollis	2500	-	-	-	-
	5000	-	GA	GA	GA
	7500	-	GA	GA	GA
A. robusta	2500	-	-	-	-
	5000	-	-	DO	DO
	7500	-	-	-	-
J. (D.) sp. cf. gemmacea	2500	-	-	RA, GA	RA, GA
	5000	-	RA, GA	RA, GA	RA, GA
	7500	-	RA, GA	RA, GA	RA, GA
C. (Verrucella) sp. A	2500	-	-	-	-
	5000	-	-	-	-
	7500	-	RA	RA	RA
Echinogorgia sp. E	2500	-	-	-	-
	5000	-	-	GA	GA
	7500	-	-	GA	GA
Control (dechlorinated water)	-	-	-	-	-

erosa, J. (D.) cf. gemmacea and *C. (Verrucella).* sp. A; disorientation from *S. suberosa* and *A. robusta* and air gulping from *S. mollis* and *Echinogorgia* sp. E. No discernible response was elicited from any test solution until after 30 minutes of exposure.

DISCUSSION

The present study utilises the strategy of several directed screens (Rinehart, 1988) to test for toxicity of crude extracts towards representative prokaryotic (bacteria) and eukaryotic

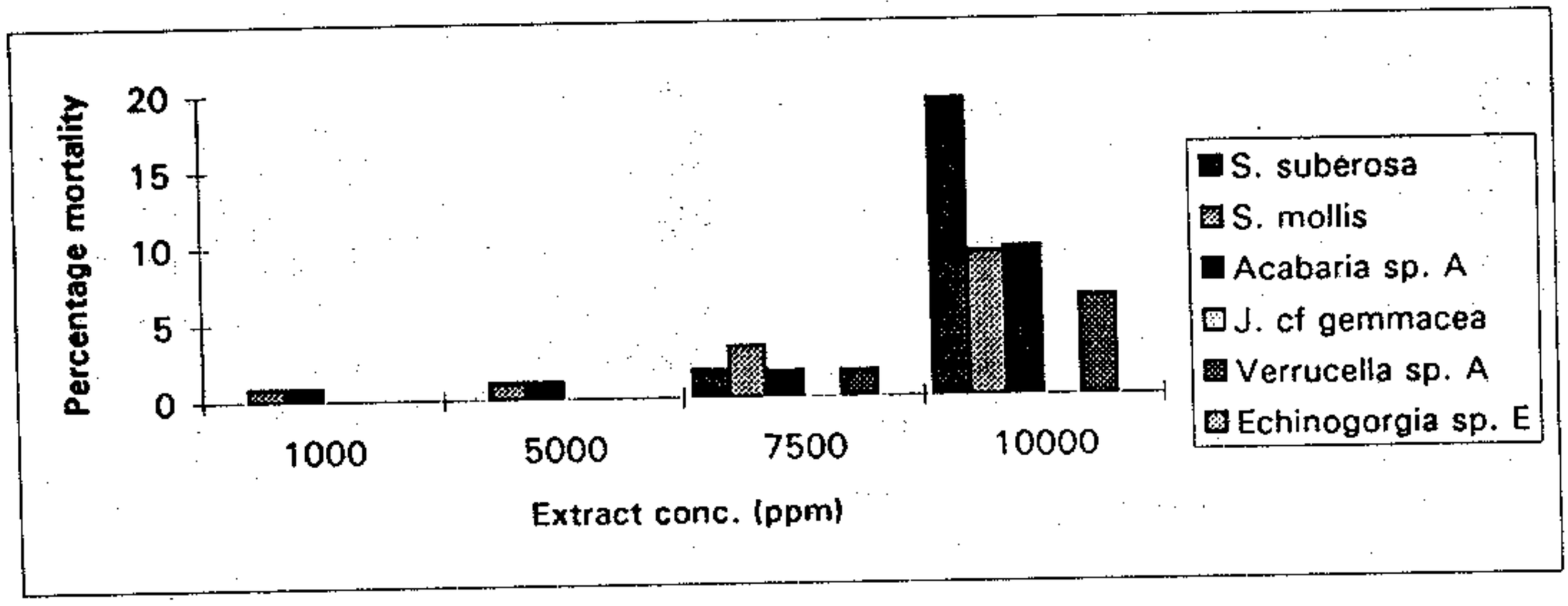

Fig. 1: Percent mortality of brine shrimp larvae after acute (6h) exposure to different concentrations of crude extracts.

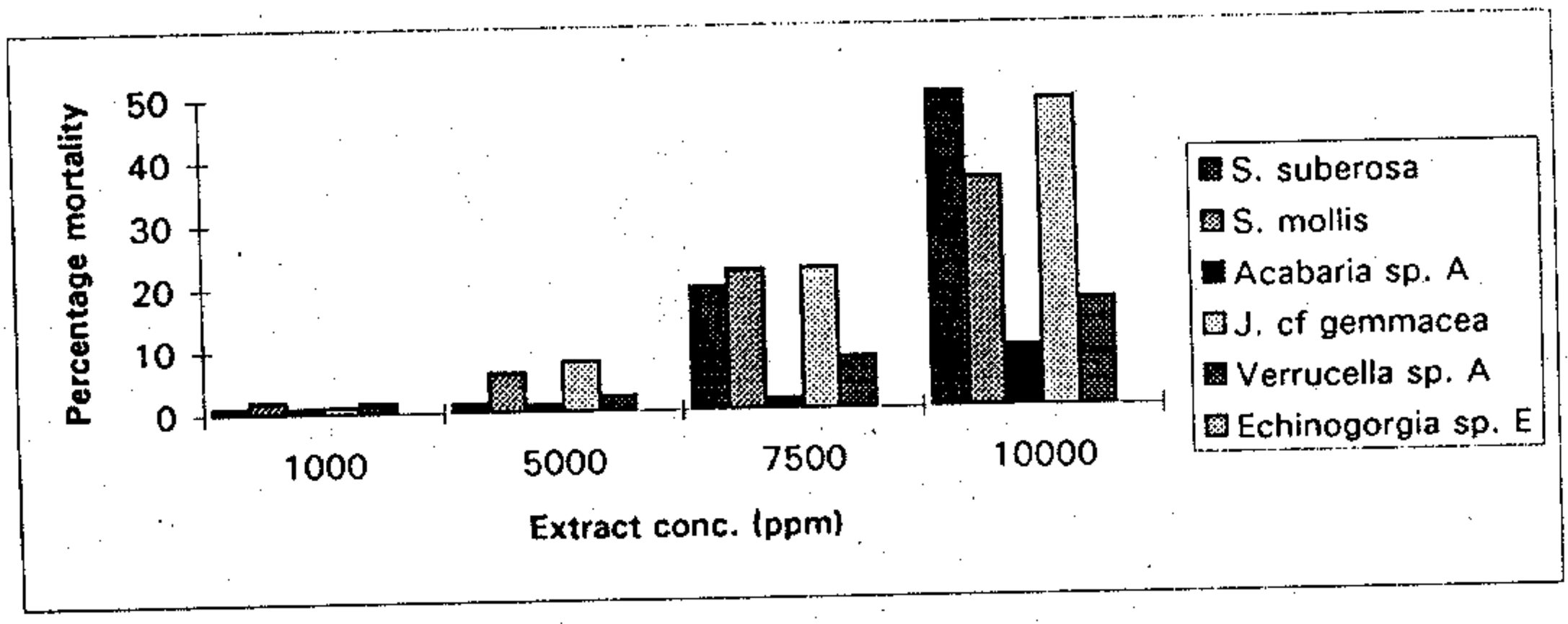

Fig. 2: Percent mortality of brine shrimp larvae after chronic (24h) exposure to different concentrations of crude extracts.

(fungi and yeast) microorganisms, invertebrates (*A. salina*), and the goldfish. Antimicrobial activity has also been used as an indication of bioactivity (Burkholder & Burkholder, 1958; Burkholder & Ruetzler, 1969; Amade *et al.*, 1982; Thomson *et al.*, 1985; Kim, 1994), as has the *A. salina* screen (McLaughlin *et al.*, 1993; Teng, 1993 and references therein). Both these assays have the advantages of being fast, cheap, simple, and without the need for very specialised

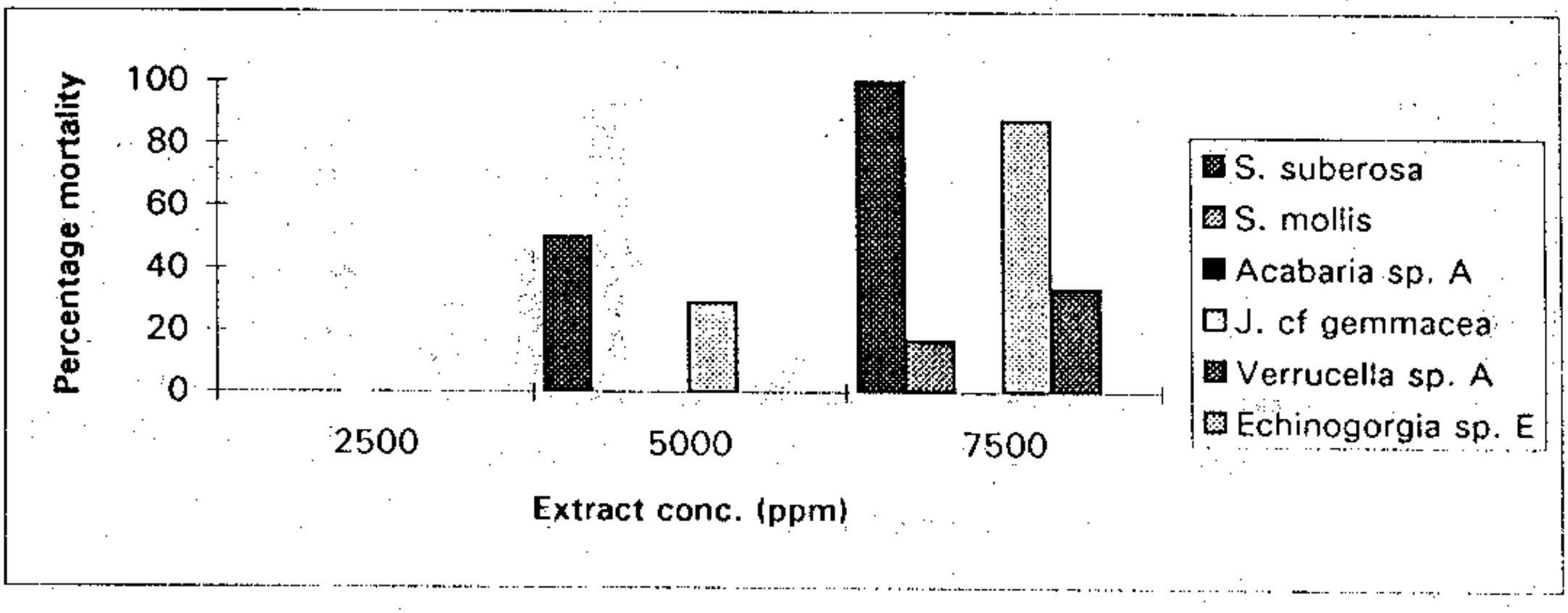

Fig. 3. Percent mortality of goldfish after acute (6h) exposure to different concentrations of crude extracts.

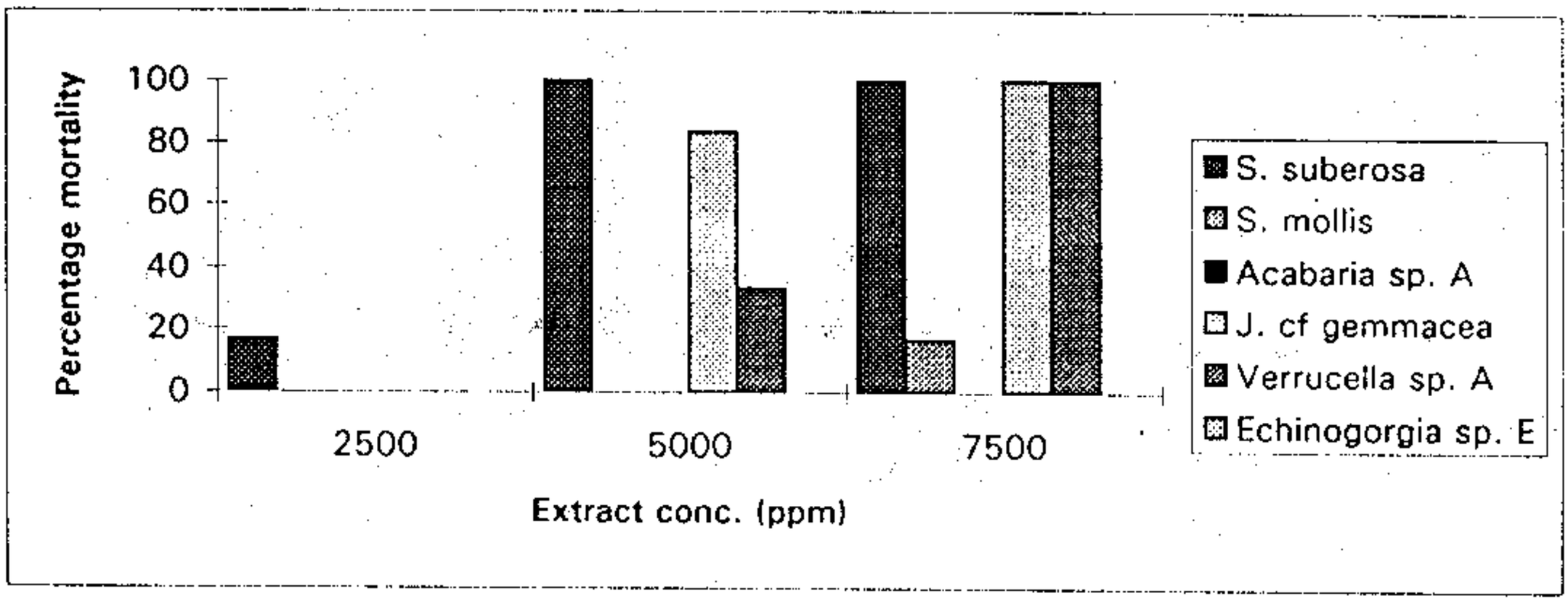

Fig. 4. Percent mortality of goldfish after chronic (24h) exposure to different concentrations of crude extracts.

equipment or training. Vertebrates possess more complex enzymatic and physiological systems than invertebrates and microorganisms, and activity against such systems would not be detected by only the first two screens. The use of goldfish as a test organism, besides easy availability, has the advantage of having been studied in terms of their susceptibility to test extracts (Thomson *et al.*, 1985). The use of the freshwater goldfish may seem illogical for testing extracts of marine origin. However, in effect, the sensitivity of the assay is increased since such a freshwater fish is unlikely to possess co-evolved resistance to gorgonian toxins or feeding deterrents (Sammarco & Coll, 1988). Toxicity tests that are negative for assays using a marine fish found naturally with gorgonians may be false due to acquired fish resistance and thus complicate the interpretation of results.

Kim (1994) reported antimicrobial activity in gorgonians from Panama (range of inhibition diameters: up to 3mm) while Thomson *et al.* (1985) tested forty species of sponges from the southern Californian coast and used two categories for scoring antimicrobial activity, i.e., those less than 2mm inhibition diameters and those with greater than 2mm inhibition diameters. Though not explicitly stated, this implies that they did not obtain inhibition diameters much larger than 4mm. Some of the extracts of temperate and tropical sponges tested by Amade *et al.* (1982) gave inhibition diameters of 2mm-12mm (mostly 5mm-12mm) against Gram-positive bacteria, and only one positive result (2mm) in 38 assays against the mould *Aspergillus*. For the Gram negative bacterium *E. coli* tested, inhibition diameters ranged from 2mm - 5mm. In contrast, when inhibitory activity was present in this study, inhibition diameters against both Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria ranged from 9mm - 12mm. In this study, the extract of *J. (D.)* sp. *cf. gemmacea* was found to be also active against the eukaryotic *S. cerevisiae* in addition to being active against *B. subtilis* and *E. coli*. Since different extraction methods, solvents and loading concentrations were used, it is not feasible to compare the results obtained here with those reported elsewhere. However, the observed antimicrobial activity in the present study does suggest the presence of bioactive compounds in gorgonian species from Singapore.

Microbial colonization is potentially pathogenic to marine invertebrates (Mitchell & Chet, 1975) and may also be the precursor of secondary macro-fouling (Baier & Meyer, 1991). The possession of anti-microbial compounds would thus be an asset to the survival and growth of a sessile marine invertebrate. It has also been observed (NKCG) that in general, secondary macro-fouling in Singapore gorgonians only occurs when the living tissue has been scraped off or abraded. This provides circumstantial evidence that bioactive compounds in gorgonians may have a natural anti-bacterial/anti-fouling role. More research is needed to substantiate this hypothesis, although work on gorgonians elsewhere (Rittschof *et al.*, 1984; Banduragga & Fenical, 1985) supports this.

Thomson *et al.* (1985) also used *A. salina* as a bioassay organism, but their results were qualitative (active or inactive) rather than quantitative, and the

solutions tested were purified metabolites rather than crude extracts. McCaffrey (1985) reported that compound A from the sponge *Callyspongia* sp. was toxic to *A. salina* at an unusually high concentration of 20 gml^{-1} (cf. 0.01gml^{-1} for 10000 ppm). If this figure is correct, many of the extracts tested here are at least two orders of magnitude more toxic. Unfortunately, no actual figures or percentages were given to define 'toxicity', so direct comparisons cannot be made. Comparison is made instead with the positive control using the heavy metal salt potassium dichromate. All extracts were less toxic than the most dilute (20 ppm) solution of potassium dichromate tested (cf. Figs.1 & 2 and Table 2), which caused almost 92% mortality. It must be remembered that the extracts are crude, and a pure heavy metal would definitely have a greater toxic effect than an extract containing a small proportion of active metabolites.

In the present study extracts of *Echinogorgia* sp. E did not cause any mortality at all concentrations tested, for both acute and chronic exposures. *J. (D.) sp. cf. gemmacea* was slow acting, being totally inactive in the acute exposure assay but very active after 24 hours, causing the highest percentage mortality at 5000 ppm and 7500 ppm, and being surpassed in toxicity at 10000 ppm by only one other extract (*S. suberosa*). *A. robusta* sp. A, on the other hand, was among the first extracts to elicit a response, but mortality in solutions of this extract did not increase between 6h and 24h.

At the lowest test concentration (2500 ppm) used in the goldfish mortality assay, only the extract of *S. suberosa* was active, but then only after chronic exposure (Figs. 3 and 4). This extract also proved to be among the most active at 5000 ppm and 7500 ppm in both acute and chronic assays. In contrast, both *A. robusta* and *Echinogorgia* sp. E. were completely non-toxic to goldfish. The extract of *S. mollis* was active only at 7500 ppm, causing 16.67% mortality after 6h, a level that remained unchanged even after 24h. *J. (D.)* sp. *cf. gemmacea* extract was active at and above 5000 ppm, with mortality increasing with exposure time. Mortality in *C. (Verrucella)* sp. A also increased with exposure time, with mortality at 5000 ppm and 7500 ppm after 6h increasing from 0% to 33.33% and from 33.33% to 100% at 24h, respectively.

All the extracts tested caused an initial change (Table 3) in goldfish behaviour, but when the results in this table are compared to the final toxicity of the extracts (Fig. 4), it appears that gulping for air is not a behaviour that is important in predicting final toxicity of the extract. Extracts from *S. mollis* and *Echinogorgia* sp. E at 5000 ppm only caused gulping for air during the first hour. However, *S. mollis* extracts tested at 7500 ppm cuased only 16.67% mortality in goldfish after 24h of exposure while no mortality was seen in *Echinogorgia* sp. E extracts. However, all extracts that caused reduced activity in the initial hour (i.e., those from *S. suberosa, J. (D.)* sp. *cf. gemmacea* and sp. *C. (Verrucella)* sp. A) also produced 100% goldfish mortality after 24h of exposure at 7500 ppm.

All six extracts tested (except that of *Echinogorgia* sp. E) possessed metabolites that were bioactive as observed in all the four major screens (antimicrobial,

invertebrate toxicity, vertebrate toxicity, goldfish behaviour modification). None of the extracts was toxic to all the individual organisms assayed, and *Junceella cf. gemmacea* was the only species that could inhibit the yeast. Besides the air-gulping response from the goldfish, the extract of *Echinogorgia* sp. E was toxic only to the bacteria *B. subtilis* and *E. coli.*

The different effects of the six extracts tested on the microorganisms, brine shrimp and goldfish point to the diversity of secondary bioactive compounds present in these gorgonians. For example, in the antimicrobial assays, extracts of *J. (D.)* sp. *cf. gemmacea* were active against the Gram negative *E. coli* (9mm inhibition diameter) while that of *C. (Verrucella)* sp. A was completely inactive (although both extracts had similar activities against *B. subtilis* and *S. cerevisiae*). Also, the extract from *Echinogorgia* sp. E inhibited bacterial growth but was inactive in screens against brine shrimp and goldfish.

It is apparent from the tests that the brine shrimps are more sensitive to the bioactive extracts than the goldfish at lower concentrations tested. The brine shrimp toxicity tests also appear to be more reliable in that increased mortality is observed with increased exposure time. These observations may be attributable to species differences in terms of susceptibility or to differences in the physiological targets of the toxins involved.

Further work needs to be carried out to isolate the active compounds and to intensify the screening of these compounds, particularly those from *S. subserosa* and *J. (D.) sp. cf. gemmacea*.

ACKNOWLEDGEMENTS

The Reef Ecology Study Team, Department of Zoology, National University of Singapore, is acknowledged for help in sample collection. NKCG is supported by a Research Scholarship from the National University of Singapore.

REFERENCES

Amade, Ph., Pesando, D. & Chevolot, L., 1982. Antimicrobial activities of marine sponges from French Polynesia and Brittany. **Mar. Biol.,** 70: 223-228.

Baier, R.E. & Meyer, A.E., 1991. Aspects of bioadhesion. pp: 407-425. In: Fundamentals of Adhesion. (L.H. Lee, ed.) Plenum Publishing.

Burkholder, P.R. & Burkholder, L.M., 1958. Antimicrobial activity of horny corals. **Science** (Washington, D.C.), 127: 1174.

Burkholder, P.R. & Ruetzler, K., 1969. Antimicrobial activity of some marine sponges. **Nature,** 222: 983-984.

Coll, J.C., 1981. Proc. 4th Asian Symp. Med. Plants and Spices. pp: 197-204. UNESCO Spec. Publ., Bangkok,

Fenical, W., 1987. Marine soft corals of the genus *Pseudopterogorgia*: a resource for novel anti-inflammatory diterpenoids. **J. Nat. Prod.,** 50: 1001-1008.

Fenical, W., Okuda, R.K., Bandurraga, M.M., Culver, P. & Jacobs, R.S., 1981. Lophotoxin: a novel neuromuscular toxin from the Pacific sea whips of the genus *Lophogorgia*. **Science,** 212: 1512-1514.

Fenical, W. & Pawlik, J.R., 1991. Defen-

sive properties of secondary metabolites from the Caribbean gorgonian coral *Erythropodium caribaeorum*. **Mar. Ecol. Prog. Ser.,** 75: 1-8.

Gerhart, D.J., 1986. Gregariousness in the gorgonian-eating gastropod *Cyphoma gibbosum:* test of several causes. **Mar. Ecol. Prog. Ser.,** 31: 255-263.

Goh, N.K.C. & Chou, L.M., 1994. Distribution and biodiversity of Singapore gorgonian (subclass *Octocorallia*) — a preliminary survey. **Hydrobiologia,** 285: 101-109.

Groweiss, A., Look, S.A. & Fenical, W., 1988. Solenolides, new anti-inflammatory and antiviral diterpenoids from a marine octocoral of the genus *Solenopodium*. **J. Org. Chem.,** 53: 2401-2406.

Harvell, C.D. & Fenical, W., 1989. Chemical and structural defense of Caribbean gorgonians (*Psedopterogorgia* spp.): intracolony localization of defense. **Limnol. Oceanogr.,** 34: 382-389.

Harvell, C.D., Greene, C.H. & Fenical, W., 1988. Chemical and structural defences of Caribbean gorgornians (*Pseudopterogorgia* spp.) 1. development of an in situ feeding assay. **Mar. Ecol. Prog. Ser.,** 49: 287-294.

Hickson, S.J., 1924. An introduction to the study of recent corals (The early trade in black land red coral, chapter XII). pp: 231-250. Publ. Univ. Manchester, Biol. Ser. No.IV.

Kennish, M.J., 1989. Practical handbook of marine science. Compounds from marine organisms (Section 9). pp: 419-689., CRC Pres, Boca Raton, Florida, USA,

Kim, K., 1994. Antimicrobial activity in gorgonian corals (Coelenterata, Octocorallia). **Coral Reefs,** 13: 75-80.

Kobayashi, J., Cheng, J.F., Nakamura, H., Ohizumi, Y., Matsuzaki, T., Grace, K.J.S., Jacobs, R.S., Kato, Y., Brinen, L.S. & Clardy, J., 1991. Structure and stereochemistry of brianolide, a new anti-inflammatory diterpenoid from the Okinawan gorgonian *Briareum* sp. **Experientia (Basel),** 47: 501-502.

Lasker, H.R., 1985. Prey preference and browsing pressure of the butterflyfish *Chaetodon capistratus* on Caribbean gorgonians. **Mar. Ecol. Prog. Ser.,** 21: 213-220.

Lasker, H.R., & Coffroth, M.A., 1988. Temporal and spatial variability among grazers: variability in the distribution of the gastropod *Cyphoma gibbosum* on octocorals. **Mar. Ecol. Prog. Ser.,** 43: 285-295.

Lasker, H.R., Coffroth, M.A. & Fitzgerald, L.M., 1988. Foraging patterns of *Cyphoma gibbosum* on octocorals: the role of host choice and feeding preference. **Biol. Bull.,** 1874: 254-266.

Look, S.A. & Fenical, W., 1987. The secopseudopterosins, new anti-inflammatory diterpene-glycosides from a Caribbean gorgonian octocoral of the genus *Pseudopterogorgia*. **Tetrahedron,** 43: 3363-3370.

Look, S.A., Burch, M.T., Fenical, W., Qi-Tai, Z. & Clardy, J., 1985. Kallolide A, a new anti-inflammatory diterpenoid, and related lactones from the Caribbean octocoral *Pseudopterogorgia kallos*. **J. Org. Chem.,** 50: 5741-5746.

Look, S.A., Fenical, W., Jacobs, R.S. &

Clardy, J., 1986. The pseudopterosins: Anti-inflammatory and analgesic natural products from the sea whip *Pseudopterogorgia elisabethae.* **Proc. Nat. Acad. Sci.** USA, 83: 6238-6240.

McCaffrey, E., 1985. Biologically active compounds from two species of marine sponges. PP: 141-146. Proc. 5th Int. Coral Reef Symp., Tahiti, Vol. 5.

McLaughlin, J.L., Chang, C.J. & Smith, D.L., 1993. Simple bench-top bioassays (brine shrimp and potato discs) for the dicovery of plant antitumor compounds. Review of recent progress. PP: 112-137. In: Human medicinal agents from plants. (A.B. King Lorn & M.F. Bolaundrin, eds.) American Chemical Society, Washington, D.C.

Mitchell, R. & Chet, I., 1975. Bacterial attack of corals in polluted seawater. **Microb. Ecol.,** 2: 227-233.

Pawlik, J.R., Burch, M.T. & Fenical, W., 1987. Patterns of chemical defense among gorgonian corals: a preliminary survey. **J. Exp. Mar. Biol. Ecol.,** 108: 55-66.

Pordesimo, E.O., Schmitz, F.L., Ciereszko, L.S., Hossain, M.B. & Van-Der-Helm, D., 1991. New briarein diterpenes from the Caribbean gorgonians *Erythropodium caribaeorum* and *Briareum* sp. **J. Org. Chem.,** 56: 2344-2357.

Rinehart, K.L., 1988. Screening to detect biological activity. PP: 13-22. In: Biomedical importance of marine organisms. (D. G. Fautin, ed.). No.13. Memoirs of the California Academy of Sciences.

Sammarco, P.W. & Coll, J.C., 1988. The chemical ecology of alcyonarian corals (*Coelenterata: Octocorallia*). PP: 87-116. In: Bioorganic marine chemistry. (P.J. Scheuer, ed.), Vol.2, Springer-Verlag.

Sammarco, P.W., La Barre, S. & Coll, J.C., 1987. Defensive strategies of soft corals (*Coelenterata: Octocorallia*) of the Great Barrier Reef: III. The relationship between ichthyotoxicity and morphology. **Oecologia,** 74: 93-101.

Scheuer, P.J., 1988. Ethno-natural leads. PP: 37-40. In: Biomedical importance of marine organisms. (D.G. Fautin ed.), No.13, Memoirs of the California Academy of Sciences.

Shin, J., Park, M. & Fenical, W., 1989. The junceelloiides, new anti-inflammatory diterpenoids of the briarane class from the Chinese gorgonian *Junceella fragilis.* **Tetrahedron,** 45: 1633-1638.

Teng, W.S., 1993. Toxicity testing using the brine shrimp: *Artemia salina*. PP: 441-456. In: Bioactive natural products. Detection, isolation and structural determination. (S.M. Colgate & R.J. Molyneux, eds.), CRC Press, Boca Raton.

Thomson, J.E., Walker, R.P. & Faulkner, D.J., 1985. Screening and bioassays for biologically active substances from forty marine sponge species from San Diego, California, USA. **Mar. Biol.,** 88: 11-21.

Tursch, B., Braekman, J.C., Daloze, D. & Kaisin, M., 1978. Terpenoids from coelenterates. PP: 247-296. In: Marine natural products; chemical and biological perspectives, vol. II, (P.J. Scheuer, ed.), Academic Press, New York.

Van Alstyne, K.L. & Paul, V.J., 1992. Chemical and structural defenses in the seafan *Gorgonia ventalina:* effects against generalist and specialist predators. **Coral Reefs,** 11: 155l-1559.

Vreeland, H.V. & Lasker, H.R., 1989. Selective feeding of the polychaete *Hermodice carunculata* Pallas on Caribbean gorgonians. **J. Exp. Mar. Biol. Ecol.,** 129: 265-277.

Weinheimer, A.J. & Matson, J.A., 1975. Crassin acetate, the principal antineoplastic agent in four gorgonians of the *Pseudoplexaura* genus. **Llyodia,** 38: 378-382.

Weinheimer, A.J. & Spraggins, R.L., 1969. The occurrence of new prostaglandin derivatives (15-epi-PGA_2 and its acetate, methyl ester) in the gorgonian *Plexaura homomalla.* **Tetrahedron Lett.,** 1969: 5185-5188.